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National Institute of Neurological  
and Communicative Disorders  
and Stroke

# Intramural Research



Annual Report  
Fiscal Year 1986

U.S. DEPARTMENT  
OF HEALTH  
AND HUMAN SERVICES  
Public Health Service  
National Institutes of Health

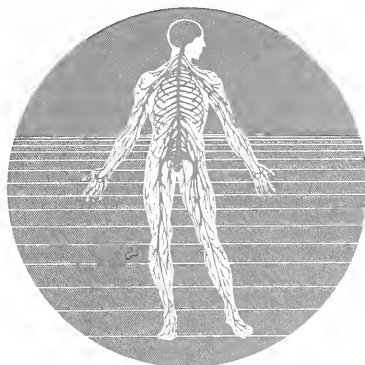
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**INTRAMURAL RESEARCH PROGRAM**

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Dr. Irwin J. Kopin, Director  
Robert N. Knickerbocker  
Administrative Officer

September, 1986

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Dr. Mark Hallett

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Dr. Ernst Freese  
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**Laboratory of Molecular Biology**  
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**Laboratory of Molecular Genetics**  
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**Laboratory of Neural Control**  
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**Laboratory of Neurobiology**  
Dr. Thomas S. Reese, Chief

**Laboratory of Neurochemistry**  
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**Laboratory of Neurophysiology**  
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**Associate Director for Branches**  
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Byron Mason  
Administrative Officer

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Dr. Thomas N. Chase, Chief

**Infectious Diseases Branch**  
Dr. John L. Sever, Chief

**Medical Neurology Branch**  
Dr. Roger J. Porter, Chief

**Neuroepidemiology Branch**  
Dr. Bruce S. Schoenberg, Chief

**Neuroimmunology Branch**  
Dr. Dale E. McFarlin, Chief

**Surgical Neurology Branch**  
Dr. Edward H. Oldfield, Acting Chief

## Annual Report of the Scientific Director

### National Institute of Neurological and Communicative Disorders and Stroke

October 1, 1985 through September 30, 1986

Irwin J. Kopin, M.D., Scientific Director

The Intramural Research Program (IRP) operates laboratories and clinics which are located mainly within the NIH campus in Bethesda. Components of some investigations are performed away from Bethesda, in laboratories at Fort Detrick in Frederick, Maryland and at the Marine Biological Laboratory in Woods Hole, Massachusetts. Federal Government scientists, their support staffs, and guest research workers continue to make new discoveries and advance our knowledge regarding means of prevention, amelioration, or cure of neurological or communicative diseases. Investigator-initiated research projects range from studies of biological processes at a molecular level to new approaches to medical or surgical therapy of neurological disorders. Such studies contribute to the rapidly expanding body of new knowledge in the neurosciences and enhance an understanding of how disease processes are initiated and progress to produce neurological dysfunction. All of these investigations advance biomedical knowledge for the ultimate prevention or alleviation of human suffering from disease or injury, which is the main mission of the Institute and of the NIH.

This year Dr. Mark Hallett, Clinical Director, NINCDS, has assumed also the position of Associate Director for Branches, NINCDS, complementing the role of Dr. Ernst Freese, who is Associate Director for Laboratories. I feel fortunate in having available two Associate Directors who are such extremely knowledgeable and capable persons. Together they provide wise counsel and valuable views and play important roles in the management of fundamental investigations in

the Laboratories and clinical studies in the Branches. Both Dr. Hallett and Dr. Freese carry on their independent research as well as serve in these important leadership roles.

This summary of FY 86 will focus on major personnel changes, space reallocations, and budget of the IRP, whereas summaries of the major scientific advances in the Laboratories and Branches are presented in the reports contributed by the individual Laboratory and Branch Chiefs.

#### Personnel Issues

During the last year, the number of employees in the IRP has increased to ceiling levels, mainly due to the hiring of young investigators. At the end of August, there were 526 employees (of whom 257 occupied full time permanent slots) using a total of 449 FTE positions. There were 184 professional non-tenured employees (23 Medical Staff Fellows, 37 Staff Fellows, 51 Senior Staff Fellows, 14 Special Experts, 59 Visiting Associates or Visiting Scientists) and 147 ceiling-free positions (74 Visiting Fellows, 72 Guest Workers, and one Intergovernmental Personnel Act employee). The inauguration of a new Intramural Research Training Associate Program (IRTA) will allow research fellows with U.S. citizenship to be employed in ceiling-free positions; it is anticipated that the number of Visiting Fellows, Staff Fellows, and Medical Staff Fellows will be decreased as space becomes the limiting factor in expanding IRTA participation in the IRP laboratories.

During FY 86 there have been several departures from among the tenured NINCDS scientists. Dr. Paul Kornblith, Chief, Surgical Neurology Branch (SNB), left to assume the chairmanship of a university department of neurosurgery. This position presented a new challenge for Dr. Kornblith and commanded a salary about three-fold greater than the highest salary NIH could offer. Dr. John Barranger, Chief, Clinical Investigations and Therapeutics Section,

Developmental and Metabolic Neurology Branch, became professor of pediatrics at a medical school in California which could offer new clinical opportunities as well as a substantial increment in salary. Dr. Edward Ginns, who worked closely with Dr. Barranger, accepted a research position with NIMH. Dr. John Kebabian and Dr. Tom O'Donohue, both Ph.D.'s with excellent records of neuropharmacology investigations, have each assumed research leadership positions with two different pharmaceutical firms at salaries in excess of the highest NIH salary. Recruitment of suitable replacements for these important contributors to our research program is actively underway. It is likely that younger, promising investigators will be selected since it is unrealistic to believe that active, established investigators can be convinced to interrupt their research programs to join a Government service which offers substantially lower salaries. However, the excellent scientific environment at NIH offers unique opportunities for research training and experience for younger investigators to achieve independence and establish reputations. The accomplishments of NIH have always included the professional development of scientists in research skills as well as the advancement of scientific information. However, we have been fortunate in the recruitment from NICHD of Dr. Harold Gainer as the new Chief, Laboratory of Neurochemistry. Dr. Gainer has an established reputation as a superb neuroscientist. The opportunity to develop a new research program offered by a switch to NINCDS, as well as the association with other NINCDS scientists having complementary research interests, provided the major impetus for Dr. Gainer to join the NINCDS, IRP. We hope to establish Dr. Gainer in fully operational research laboratories within a few months.

Salary differentials have also had a major impact on the ability of the Clinical Center to retain or recruit nursing staff. Because of understaffing of the nursing units of NINCDS, in the interests of patient safety, Dr. Hallett, the Clinical Director, has found it necessary to diminish the patient census and to place limitations on

the numbers of patients admitted by each of the Branches. The implementation of a new, higher, salary scale for nurses, based on current salaries in the Veterans Administration, may help to alleviate this nursing shortage.

#### Space Issues

Space limitations and renovation delays continue to be major difficulties in the rapid implementation of new research directions and in providing suitable laboratories for scientists who are displaced in order to accommodate development of animal care facilities according to AALAC accreditation standards which has taken the highest priority. A complicated series of renovations and moves have been planned to accommodate the new facilities and to provide laboratories for NINCDS investigators who will be returning to the NIH campus from the Marine Biological Laboratory.

The reallocation of space within the NINCDS, IRP, continues to progress in spite of time slippages in completion of required renovations and scheduled moves. The NINCDS Outpatient Clinic which was to be located on the fifth floor, ACRF, is now operating in a shared facility on the seventh floor. The clinic space on the fifth floor has been used as "swing space" to accommodate offices and "dry laboratories" which were required to meet the needs of the clinical program. The space vacated by these operations is being converted to "wet laboratories" to partially meet the needs for the SNB Laboratories which will be displaced by the conversion of Building 10A to a centralized NIH animal research facility. Additional office space is being made available by using about 1000 square feet of office space on the 5D corridor. This corridor will be used to house temporarily the Blood Bank, which was previously located on the first floor of Building 10. This temporary location of the Blood Bank will cause a delay of planned moves of the NINCDS to that allocated space.

As indicated in the Annual Report last year, Building 9 is being redesigned to centralize primate housing and to ensure adequate space for surgical procedures and behavioral observations in monkeys. Dr. Herbert Amyx, Institute Veterinarian, continues to oversee for the NINCDS the development of this facility. We now plan to include in this resource primates which are currently housed in Building 36 and to have Dr. William London take charge of all primate care in this centralized NINCDS facility. As previously indicated, laboratory space in Building 9 is being consolidated to provide for a new Laboratory of Neuronal Regeneration and Implantation. This laboratory has been approved and a search committee was established to search for a key Chief. But prior to the initiation of this search, approval is necessary to advertise the position in the SES.

During the last seven to ten years the NINCDS has maintained at the Marine Biological Laboratory (MBL) at Woods Hole, laboratories which operated during the entire year as well as additional laboratories which were used only during the summer for special studies requiring fresh squid. The senior investigators in these laboratories, Dr. William Adelman, Chief, Laboratory of Biophysics; Dr. Daniel Alkon, Chief, Section on Neural Systems in the Laboratory of Biophysics; and Dr. Thomas Reese, Chief, Laboratory of Neurobiology, have conducted important research. However their distant location from the NIH campus has limited their association with the NINCDS investigators and their contributions to the intellectual critical mass of the IRP. While year-round laboratories at the MBL appeared to be useful for time-limited research efforts of their parent laboratories, and excellent research has been accomplished, it has become clear that it is now time for these investigators to establish closer ties to the NIH campus if they are to be a meaningful part of the IRP research community.

The space required for the return to the NIH campus of the MBL senior investigators has necessitated expanded use of the Park Building (a short distance from the NIH campus). Additional space in Building 36 is being made available by the relocation of some laboratories to Building 9. The increased allocation by the NIH of space rented in the Park Building has provided the opportunity to build a new laboratory (to be named) composed of four independent investigators who have complementary research interests based in biochemistry. This laboratory will have an annually rotating laboratory Chief. Each of the sections will have a separate budget and identified space and personnel. Some additional space and funds will be made available for the Acting Laboratory Chief to administer the laboratory and apply to extraordinary needs. Dr. Daniel Alkon is to be one of these four Section Chiefs; Dr. Richard Quarles, Chief, Section on Myelin and Brain Development, Developmental and Metabolic Neurology Branch (DMNB), who is presently located in the Park Building will be the second; and Dr. Peter Fishman, Chief, Membrane Biochemistry Section, DMNB, is the third. Both Dr. Quarles and Dr. Fishman have developed independent lines of research and Dr. Brady, Chief, DMNB, supports the view that it would be appropriate for these senior investigators to assume the responsibilities of a laboratory Chief. Dr. Craig Venter, Chief, Section on Receptor Biochemistry, LNP, (with the agreement of Dr. Barker, Chief, LNP) is to be the fourth rotating Chief of this new laboratory. It is anticipated that the combining of these four senior investigators will result in a strong new laboratory with a critical intellectual mass sufficient to offset the disadvantage of their location somewhat removed from the NIH campus. Plans for a new neuroscience building to be built on the NIH campus include space for these investigators to return to laboratories on the NIH campus, but this will require 5-7 years.

In Building 36, plans for the centralization of animal care facilities are proceeding. Laboratory space for Dr. Thomas Reese and



Dr. William Adelman will be made available by the following: the move of Dr. William London and his primate facility to Building 9; the conversion to laboratories of locker rooms in the 5C corridor; the move of Dr. Gajdusek's records to the space made available in the National Library of Medicine; and the acquisition of Dr. Venter's space when he moves to the Park Building.

#### Fiscal Issues

This has been a lean year compared to FY 85. Less total funds were available to the IRP (47.4 million versus 48.8 million in FY 85) and personnel costs rose (18.7 million versus 17.8 million in FY 85). Although the NINCDS contribution to the NIH management fund appeared to have decreased by nearly 0.5 million dollars (15.13 compared to 15.62 in FY 85), this was due to a shifting in accounting practices such that some costs previously included in the management fund are now billed directly by the Clinical Center to the Institutes and paid from the Other Objects funds. The decline in available Other Objects funds, from 15.38 million in FY 85 to 13.58 million in FY 86, is therefore even greater than the 1.8 million difference (11.7% decrement) between FY 86 and FY 85. The decrement in available funds has necessitated the postponement until FY 87 the purchase of important new equipment.

There have been several other administrative changes in the IRP during FY 86.

The Office of the Clinical Director, which has the responsibility for all matters relating to patient care, educational activities, and services provided by the NINCDS to the Clinical Center and other Institutes, has been enlarged. In addition to providing EEG, EMG, and neuropathology services and neurological consultations, the Audiology Unit, (formerly with the Clinical Center) was transferred

this year to this Office in NINCDS and will continue to provide this service.

The Developmental and Metabolic Neurology Branch has been undergoing major restructuring, particularly with regard to personnel changes. Dr. Roscoe Brady continues his major research efforts in the area of hereditary metabolic disorders. Dr. Norman Barton is assuming a leadership role in supervising the clinical studies and Dr. Brady has recruited Dr. Stefan Karlsson as a Visiting Associate, to replace Dr. Edward Ginns. With the transfer of Dr. Ginns to NIMH and the loss of Dr. Barranger, a number of research projects have been terminated. New projects are being planned which will exploit the talents of the new staff members. As indicated earlier, two of the Section Chiefs in this laboratory, who have become senior independent investigators, will join the new laboratory in the Park Building. This will enable Dr. Brady to have additional flexibility in following new directions in his research as well as increase the independence and responsibilities of these scientists.

The Experimental Therapeutics Branch is actively seeking replacements for Dr. John Kebabian and Dr. Tom O'Donohue. To maintain productivity of research, the research programs are continuing under the aegis of Acting Chiefs who have been involved in the projects of the departed investigators.

The Surgical Neurology Branch is currently being headed by Dr. Edward Oldfield, Acting Chief. The departures of Dr. Paul Kornblith and Dr. Elizabeth Grimm, who moved with her husband to Texas, have resulted in some changes in the research program as described by Dr. Oldfield. Dr. Richard Webber has been recruited to head the Neuroimmunology Unit and continue some of the work with LAK cells. The displacement of the SNB laboratories from Bldg. 10A will result in disruption of research unless adequate labs can be prepared to receive the equipment and personnel. Renovations of space on the 4N

corridor are to begin shortly, and we hope that they will be completed sufficiently soon to allow an orderly transfer with minimal loss of productivity.

The Medical Neurology Branch has completed a portion of its renovation plans and, thus, will be able to increase its research activities. The new, active Section on Neuronal Excitability is functioning well and several younger investigators have joined this laboratory. During the next year, it is hoped that renovations will be completed, but additional moves may be required to make optimal use of areas which can be converted to "wet laboratories."

The Neuroepidemiology Branch continues to be located in the Federal Building. Dr. Karin Nelson, from the Extramural Program has recently joined this Branch and will be pursuing studies in pediatric neurology.

The Neuroimmunology Branch has completed renovations for its new laboratory. Dr. William Biddison will be Chief of a new section and has embarked on studies involving DNA sequencing to investigate the molecular basis of cellular immunological resistance.

The Neuroimaging Section in the Office of the Director, headed by Dr. Giovanni Di Chiro, continues to effectively serve as the focal point for NINCDS interaction with the Nuclear Medicine and Radiology Departments in the Clinical Center. The PET and MRI programs are developing satisfactorily and the research efforts of these groups are among the foremost in the world.

The Laboratory of Biophysics, as indicated above, has plans underway relating to the IRP not maintaining its year-round operations at Woods Hole. Space is being prepared for the transfer of these operations to the NIH campus. Similarly, space is planned for the

return of the portion of the Laboratory of Neurobiology located at MBL.

In order to implement the creation of the new Laboratory of Neuronal Regeneration and Implantation (LNRI), two Sections presently headed by Dr. Kopin and Dr. Zalewski will be transferred to the LNRI. Plans for allocation of required space and FTE positions will be completed before the position for Chief of this new laboratory is advertised.

In summary, in FY 86 the Intramural Research Program continues to be characterized by successful accomplishments despite the above mentioned constraints. We hope that in FY 87 a substantial number of renovations can be completed, planned moves accomplished, and the Chief positions recruited for the SNB and the LNRI. Accomplishing these goals are critical elements in the forward progress of the IRP.





## ANNUAL REPORT

October 1, 1985 through September 30, 1986

Office of the Clinical Director  
Office of the Director, Intramural Research Program  
National Institute of Neurological and Communicative Disorders and Stroke

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## Annual Report: October 1, 1985 to September 30, 1986

### Office of the Clinical Director

Mark Hallett, M.D., Clinical Director

The Office handles administrative matters, mainly relating to patient care, coordination of educational activities, and delivery of neurological services. Service functions can be divided into the EEG Laboratory, the EMG Laboratory, the Consultation Service, Neuropathology and Paraprofessional Support Services. In this year we have added the Audiology Unit, which had been a part of the Clinical Center, and supervision of ENT consultation.

The structural changes have been made to the fifth floor of the ACRF for permanent homes of the NINCDS service laboratories. The outpatient clinic will also be on this floor eventually, but the move will be postponed because of space and personnel restrictions at the Clinical Center. The initial programming for the renovations of the inpatient facilities on 5E and 5W have been completed. The two major educational conferences are the Clinical Conference (held on Tuesday afternoon), which is aimed at the Medical Staff Fellows and typically reviews a patient in detail, and the NINCDS Grand Rounds (held on Friday afternoon). The Clinical Conference includes some attention to matters of patient care and quality assurance. The Grand Rounds continues to offer CME credit.

**EEG Laboratory, Susumo Sato, M.D., Chief**

#### Diagnostic Services:

The total number of tests performed during this reporting period increased by approximately two hundred for the EEG examination and remained at about the same level for evoked potential tests (EP) compared to the last reporting period. The major portion of the increase came from NIMH and NCI.

	<u>EEG</u>	<u>Evoked Potentials</u>
NINCDS	384	256
NINCDS OPD	313	76
NICHHD	40	3
NIMH	170	0
NCI	101	5
NHLBI	34	17
NIAID	34	8
CC	16	1
Normal Volunteers	0	29
Total (1712)	1308	404

#### Participation in Research Activity:

The EEG laboratory maintains a close tie with the Epilepsy Section of the Medical Neurology Branch and provides an invaluable diagnostic service in terms of localization of epileptiform discharges. The laboratory is capable of video recording events during the routine EEG recording and this capability often provides an invaluable observation in the research endeavors. The laboratory personnel prepare patients for PET scanning and

magnetoencephalographic recording. The latter has been rather frequent. The laboratory personnel also assist in preparing the patients for, and in monitoring, subdural electrode recording, electrocorticographic recording during temporal lobectomy, intracarotid sodium amytal injection (Wade test) for locating the dominant hemisphere for speech, and sphenoidal electrode recording in patients with epilepsy.

The laboratory personnel also prepare test subjects for the psychology group, for their evoked potentials.

Patients with multiple sclerosis, who participate in the treatment of cyclosporine, have been the major referrals for evoked potential testings. Evoked potentials also were done in familial Alzheimer patients.

The EEG laboratory provides a training environment for a Medical Staff Fellow toward the American EEG Board. The laboratory chief continues to serve as associate examiner for the Board.

**EMG Laboratory, Mark Hallett, M.D., Acting Chief**

EMG ACTIVITIES (July 1, 1985 - June, 30, 1986)

Referrals	NINCDS	71
	Other Institutes	64
Collaborative study	Post-polio syndrome	25
	Dysautonomia	15
	Fabry's Disease	3
	Polymyositis	14
Protocol 84-N-203	Diagnostic dilemmas	9
	Normals	7
TOTAL		<u>208</u>

Dr. Hallett has continued running the laboratory on an interim basis for most of the year. Dr. Jacob Meer arrived in July 1986 to run the laboratory temporarily for one year until Professor Roger Gilliatt comes in the summer of 1987 to serve as permanent chief. Professor Fritz Buchthal has continued to visit the laboratory frequently to consult, advise, teach and participate in research activities. The laboratory takes referrals, is participating in a number of collaborative investigations, and conducts some independent research.

Extensive studies of autonomic nerve function have been carried out in normal subjects, and patients with autonomic failure from either idiopathic orthostatic hypotension (IOH) or multiple system atrophy (MSA). It is possible to distinguish normals from patients on the basis of several parameters, but it has not been possible to separate the two groups of patients.

Detailed studies have been carried out in patients with post-polio muscular atrophy (PPMA) syndrome. Methods included nerve conduction, repetitive stimulation, quantitative EMG and single fiber EMG. There were marked abnormalities of routine and single fiber EMG, but not much difference between patients with PPMA and patients with a past history of polio, but without PPMA.

Publications of the laboratory are listed with the project reports.

The neurology consultation service provides emergency and routine consultations for patients hospitalized in the Clinical Center and outpatients referred to the Ambulatory Care Research Facility. In 1985, a total of 750 patients were evaluated. From July 1985 through June 1986, 575 patients were seen. Outpatients are referred to the bi-weekly neurology clinic or evaluated in other speciality clinics (oncology, hematology, surgery). As a part of the consultation service, we facilitate the performance of necessary diagnostic procedures in other departments (i.e., myelography, electromyography, head scans), and arrange neurological follow-up as indicated.

#### Publications:

Wichman, A., Oldfield, E.O., Pescovitz, O.H., and Cutler, G.: Precocious puberty and CNS lesions. Abstract Neurology 36 (4, suppl. 1), 150, 1986. Presented at the 38th Annual Meeting of the American Academy of Neurology, New Orleans, April 1986.

Dr. Marinos Dalakas has performed muscle and nerve biopsies in the operating room under local anesthesia for the investigation of neuromuscular symptoms of patients from different Institutes. The biopsied specimens are processed in the histochemistry laboratory he has established, for a battery of histochemical reactions. He has also processed or reviewed several muscle biopsy specimens sent to us from outside hospitals for expert advice. He has also seen, in consultation, several patients with neuromuscular complaints, admitted and studied by investigators of other Institutes. He has been studying patients under approved clinical protocols in collaboration with investigators in other Institutes. Progress in his research activities is reported with the Infectious Disease Branch, NINCDS.

#### Neuropathology, David A. Katz, M.D.

Diagnostic neuropathology services for NINCDS, and for all other Institutes, are provided by Dr. Katz. The neuropathology service is integrated with both the Autopsy and Surgical Pathology Sections and residency training program of the Laboratory of Pathology, NCI; a high priority is given to resident teaching. The brain is examined in approximately 100 of the 150 autopsies performed at NIH each year; fully 25% of these manifest significant primary or secondary neurological disease. Braincutting, held weekly, is scheduled so as to encourage participation by interested physicians and nurses. Relevant neuropathological findings are also presented at gross autopsy conferences. Selected cases are further utilized for neurological clinical conferences. Neurosurgical specimens include both in-house and submitted material, for an annual total of approximately 175 cases; intra-operative frozen-section consultations are required in approximately 35 in-house cases per year.

The neuropathology service also functions in a collaborative manner to provide subspecialty expertise in a range of clinicopathologic investigations. Current collaborative activities include: (1) participation in Alzheimer's disease protocols from both NIMH (Trey Sunderland, M.D.) and NIA (Stanley Rapoport, M.D.); (2) study of brain biopsies from patients with suspected progressive multifocal leukoencephalopathy (PML) (Sidney Houff, M.D., Ph.D.); (3) review of biopsy material from patients with precocious puberty (Alison Wichman, M.D.); (4) correlation of pathological and neuroimaging data in temporal lobectomy specimens from epileptic patients (William Theodore, M.D.). Also in progress is the formulation of formal Clinical Center protocols for diagnostic brain biopsy and for appropriate precautions regarding suspected cases of Creutzfeldt-Jacob disease.

### **Paraprofessional Support Services**

Linda Nee, MSW, is assigned to the Clinical Neuropharmacology Section, Medical Neurology Branch. She has been pursuing clinical and family studies, organizing field clinics and undertaking genetic counselling.

Helen Krebs, RN, is assigned to the Neuroimmunology Branch, where she is taking a major role in running a clinical trial of the use of cyclosporin in multiple sclerosis.

Marjorie Gillespie, RN, is assigned to the Experimental Therapeutics Branch, where she supports several aspects of the clinical program.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 NS 02675-02 ODIR
<b>PERIOD COVERED</b> October 1, 1985 through September 30, 1986		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) Evaluation of Neuromuscular Diseases		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Mark Hallett, M.D.      Clinical Director	OCD   ODIR   IRP   NINCDS
Others:	John Ravits, M.D.      Medical Staff Fellow	OCD   ODIR   IRP   NINCDS
	Michael Baker, M.D.      Medical Staff Fellow	OCD   ODIR   IRP   NINCDS
	Marinos Dalakas, M.D.      Senior Staff Fellow	OCD   ODIR   IRP   NINCDS
<b>COOPERATING UNITS</b> (if any) None		
<b>LAB/BRANCH</b> Office of the Clinical Director, Office of the Director, Intramural Research Program		
<b>SECTION</b> EMG Laboratory		
<b>INSTITUTE AND LOCATION</b> NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	0.5	PROFESSIONAL: 0.4      OTHER: 0.1
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)  Understanding of <u>neuromuscular disease</u> is founded on careful clinical observation, <u>electrodiagnostic studies</u> and <u>pathology</u> . This protocol has been carried out to learn more about established diseases, to characterize new diseases, to assess current methodologies and technologies and to refine old methods and develop new ones.  Extensive studies of <u>autonomic nerve function</u> have been carried out in normal subjects, and patients with <u>autonomic failure</u> from either <u>idiopathic orthostatic hypotension</u> (IOH) or <u>multiple system atrophy</u> (MSA). It is possible to distinguish normals from patients on the basis of several parameters, but it has not been possible to separate the two groups of patients.  Detailed studies have been carried out in patients with <u>post-polio muscular atrophy</u> (PPMA) syndrome. Methods included nerve conduction, repetitive stimulation, quantitative EMG and single fiber EMG. There were marked abnormalities of routine and single fiber EMG, but not much difference between patients with PPMA and patients with a past history of polio, but without PPMA.		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 NS 02676-02 ODIR
<b>PERIOD COVERED</b> October 1, 1985 through September 30, 1986		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) Thoracic and Abdominal Somatosensory Evoked Potentials in Normal Volunteers		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Mark Hallett, M.D. Clinical Director	OCD ODIR IRP NINCDS
Other:	Michael Baker, M.D. Medical Staff Fellow	OCD ODIR IRP NINCDS
<b>COOPERATING UNITS</b> (if any) None		
<b>LAB/BRANCH</b> Office of the Clinical Director, Office of the Director, Intramural Research Program		
<b>SECTION</b> EMG Laboratory		
<b>INSTITUTE AND LOCATION</b> NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	0.1	PROFESSIONAL: 0.1 OTHER: 0.0
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.) <p>The purpose of this study was to establish normal values for <u>somatosensory evoked potentials</u> evoked by stimulation of the skin over the anterior surface of the thorax and abdomen. This has been accomplished and the project is terminated at the end of this reporting period. The findings should be useful for evaluation of patients with <u>spinal cord dysfunction</u>.</p>		







ANNUAL REPORT  
October 1, 1985 through September 30, 1986

Neuroimaging Section, OD, IRP  
National Institute of Neurological and Communicative Disorders  
and Stroke

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ANNUAL REPORT  
October 1, 1985 through September 30, 1986  
Neuroimaging Section, ODIR, IRP  
National Institute of Neurological and Communicative  
Disorders and Stroke

Giovanni Di Chiro, M.D., Chief

SUMMARY

Following is a summary of the major findings for the research protocols of the Neuroimaging Section in the fiscal year October 1, 1985 through September 30, 1986.

Radiographic and Radioisotopic Angiography of the Spinal Cord.

(Project #Z01 NS 01195-22) Angiographic studies of arteriovenous malformations and vascular tumors of the spinal cord have continued. Digital subtraction angiography (DSA), either intravenous or intraarterial, has not proven to be particularly reliable. More useful, at least for the recognition of the vascular nature of these lesions, has been the technique of dynamic computed tomography (DCT). The possibilities and limits of magnetic resonance imaging (MRI) in the diagnostic assessment of these lesions have also been delineated (see following protocol).

Computed Tomography (Transmission) and Nuclear Magnetic Resonance (NMR). (Project #Z01 NS 02073-13) CT studies of such conditions as degenerative diseases of the CNS, cavities of the brain stem and spinal cord, and brain and spinal cord tumors and malformations have continued.

The NMR imaging research has developed along several lines:

- 1) Taking advantage of the exquisite MRI display of morphological detail to advance the diagnostic yield in a number of neurological lesions.
- 2) Carrying out a study of a large group of patients with tumors and arteriovenous malformations of the spinal cord. This study represents the first assessment of MRI capabilities in one area of spinal cord pathology. MRI has proven of some use in: 1) differentiation between tumoral versus syringomyelic cord cavities; and 2) recognition of the intramedullary location of the nidus of some arteriovenous malformations.
- 3) Trying to learn more about the NMR signal of various abnormal tissues. Particular attention has been devoted to the signal from CNS tumors of various types and grades and from extravasated, intracranial blood. We are also engaged in a comparative "in vivo/in vitro" study of T<sub>1</sub> and T<sub>2</sub> of normal and pathological CNS tissues.

- 4) Comparing clinical MRI imaging results with those of CT and particularly PET in a variety of abnormal conditions, starting with CNS tumors.
- 5) Developing an experimental method (monkeys) for MRI cisternography and myelography using Gd-DTPA. MRI cisternography has been used successfully to demonstrate cerebrospinal fluid rhinorrhea in dogs.
- 6) Assessment of NMR signal changes in the CSF cavities related to pulsatile CSF flow.

Positron Emission Tomography. (Project #Z01 02315-09) The experience with FDG-PET of primary (gliomas) CNS tumors has continued to expand. About 300 patients have been studied and in many cases repeat examinations have been performed. The usefulness of the FDG-PET for cerebral tumors is well established. This technique has also been used to predict the survival rate of patients with high grade gliomas. FDG-PET is by far the best method to establish the prognosis in these patients. The FDG-PET method of evaluation has recently been extended from the gliomas to other intracranial tumors, particularly meningiomas. Preliminary results indicate that FDG-PET is an excellent method to predict "post-removal" recurrence of meningiomas. Studies on the diagnostic reliability of FDG-PET in cases of radiation necrosis are also continuing.

An analysis of the cortical glucose metabolism in the hemianopsias starting with the homonymous field defects has been initiated. In cases of hemianopsia, the appropriate primary and associative visual cortices show marked hypometabolism.

A long range research project to compare PET with NMR - in tumors, epilepsy, and degenerative diseases - has begun. Preliminary observations indicate that the two techniques complement each other.

A "split" or "test-retest" FDG-PET method has been developed. This method permits us to assess "baseline" regional (or global) glucose utilization rates of the brain with "post-stimulation" (motor-sensory, drug-induced) values.

A study of the correlation between glucose utilization ratio and brain size. The metabolic rate per unit volume has been found to be inversely proportional to brain size.

Finally, PET studies of dopamine and opiate receptors have been carried out in a group of monkeys. Dopamine receptors have been analyzed with ( $^{11}\text{C}$ )3-N-methylspiperone and opiate receptors with ( $^{18}\text{F}$ )3-acetylcyclofxy. It appears that the anatomo-functional distribution of these ligands in the thalami and basal ganglia is different and distinctive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01195-22 ODIR

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Radiographic and Radioisotopic Angiography of the Spinal Cord

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Giovanni Di Chiro, M.D., Chief, Neuroimaging Section, ODIR, NINCDS

OTHERS:

J. L. Doppman, M.D.	Chief	DRD, CC
E. H. Oldfield, M.D.	Senior Staff Physician	SN, NINCDS
S. M. Larson, M.D.	Chief	NM, CC

COOPERATING UNITS (if any)

Diagnostic Radiology, and Nuclear Medicine Departments, Clinical Center, NIH;  
Medical Examiner's Office, Department of Public Health, Philadelphia, PA

LAB/BRANCH

Office of the Director, IRP

SECTION

Neuroimaging Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Selective arteriography (radiographic) of the spinal cord is a diagnostic technique which has proven to be informative in cases of arteriovenous malformation (AVM), tumor, obstructive vascular disease, trauma, and postradiation damage of the spinal cord.

Radioisotope angiography of the spinal cord offers some advantages as a screening method, and in certain types of intraspinal pathology may give information not available by any other diagnostic test.

Experience with the techniques of dynamic computed tomography, (DCT), digital subtraction angiography (DSA), positron emission tomography (PET) using  $^{18}\text{F}$ -2-deoxyglucose and nuclear magnetic resonance imaging (MRI) of the spine indicates that these methods may be useful screening and follow-up procedures in the evaluation of certain vascular lesions and tumors of the spinal cord.

19- ODIR/IRP (NIS)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER ZO NS 02073-13 ODIR
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Computed Tomography (Transmission) and Nuclear Magnetic Resonance (NMR)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Giovanni Di Chiro, M.D., Chief, Neuroimaging Section, ODIR, NINCDS OTHERS: R. A. Brooks, Ph.D. Staff Physicist NIS, NINCDS D. S. Fishbein, M.D. Staff Fellow NIS, NINCDS M. J. Dietz, M.D. Medical Officer NIS, NINCDS J. L. Doppman, M.D. Chief DRD, CC S. M. Larson, M.D. Chief NM, CC		
COOPERATING UNITS (if any) Diagnostic Radiology, and Nuclear Medicine Departments, Clinical Center, NIH		
LAB/BRANCH Office of the Director, IRP		
SECTION Neuroimaging Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.7	PROFESSIONAL: 1.7	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  Computed Tomography in its transmission (CT), emission (PET, SPECT), and Nuclear Magnetic Resonance (NMR) modalities, represents the main research area of the Neuroimaging Section.  <u>CT:</u> Ongoing clinical and experimental research projects in transmission CT, include studies of tumoral, degenerative, demyelinating and atrophic processes of the brain, plus hydrocephalus, brain edema, postradiation necrosis, and surgically correctable lesions in young patients affected by chronic epilepsy.  <u>NMR:</u> Our NMR imaging research is developing along six main lines: 1) we are taking advantage of the exquisite capability of NMR to display fine anatomical detail to advance our diagnostic yield in a number of neurological lesions; 2) we are trying to learn more about the NMR signal from extravasated blood (various types of CNS hemorrhages), and the signal from various types-grades of CNS tumors; 3) we are comparing our clinical NMR imaging results (emphasis on spinal cord diseases, brain tumors, degenerative diseases, and complex partial epilepsy), with those of CT and particularly PET in a variety of abnormal conditions; 4) analysis of variations in signal intensities related to pulsatile cerebrospinal fluid (CSF) flow effects; 5) studies on contrast media, e.g., Gd-DTPA, to be introduced either systemically or intrathecally; and 6) developing new NMR imaging strategies.		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02315-09 ODIR
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Positron Emission Tomography		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Giovanni Di Chiro, M.D., Chief, Neuroimaging Section, ODIR, NINCDS OTHERS: <div style="display: flex; justify-content: space-between;"> <div style="width: 60%;">           R. A. Brooks, Ph.D. Staff Physicist            D. S. Fishbein, M.D. Staff Fellow            M. J. Dietz, M.D. Medical Officer            E. J. Finn, Ph.D. Staff Physicist            *         </div> <div style="width: 35%;">           NIS, NIN            NIS, NINCDS            NIS, NINCDS            NIS, NINCDS         </div> </div>		
COOPERATING UNITS (If any) NM, CC, NIH; SN, NINCDS; MN, NINCDS; IR, NINCDS; BEIB, NIH; DRD, CC, NIH; LCM., NIMH.		
LAB/BRANCH Office of Director, IRP		
SECTION Neuroimaging Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 2.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Positron Emission Tomography (PET) using (<sup>18</sup>F)-2-deoxyglucose (FDG) allows us to obtain anatomical data (e.g., axial transverse or coronal images of the brain) as well as dynamic functional data (such as regional cerebral glucose consumption rate, and measurement of storage, degradation and turnover of tagged metabolites). Besides FDG, other radiopharmaceuticals (e.g., those tagged with <sup>15</sup>O, <sup>11</sup>C, <sup>13</sup>N) can be used with PET to study the BBB, oxygen metabolism, protein synthesis, and neuroreceptors. The unique property of PET is that it provides <u>physiologic</u> and <u>pathophysiologic</u> information not available with any other imaging procedure.</p> <p>Since June, 1982 we have been using a high-resolution, high-sensitivity scanner built in our Section, the Neuro-PET. The performance of this scanner has exceeded all our expectations. This device has made possible new applications of the PET technique.</p> <p>-----</p> <p>*Continued:</p> <div style="display: flex; justify-content: space-between;"> <div style="width: 60%;">           S. M. Larson, M.D. Chief            W. H. Theodore, M.D. Neurologist            E. H. Oldfield, M.D. Staff Physician            D. Wright, M.D. Staff Physician            C. V. Kufta, M.D. Staff Physician            M. Halleck, M.D. Clinical Director            R. J. Polinsky, M.D. Staff Physician            J. L. Doppman, M.D. Chief            L. Sokoloff, M.D. Chief         </div> <div style="width: 35%;">           NM, CC            MN, NINCDS            SN, NINCDS            SN, NINCDS            SN, NINCDS            IRP, NINCDS            MN, NINCDS            DRD, CC            LCM, NIMH         </div> </div>		
21- ODIR/IRP (NIS)		

Publications:

1. Di Chiro, G., Brooks, R.A., Bairamian, D., Patronas, N.J., Kornblith, P.L., Smith, B.H., and Mansi, L.: Diagnostic and Prognostic Value of Positron Emission Tomography Using (<sup>18</sup>F) Fluorodeoxyglucose in Brain Tumors. In Positron Emission Tomography, Reivich, M., Alavi, A. (Eds):, Alan R. Liss, Inc. NY, 1985, pp. 291-309.
2. Larson, S.M., and Di Chiro, G.: Comparative anatomic-functional imaging of two neuroreceptors and glucose metabolism. J Comput Assist Tomogr. 9(4):676-681, 1985.
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10. Di Chiro, G., Doppman, J.L., Dwyer, A.J., Patronas, N.J., Knop, R.H., Bairamian, D., Vermess, M., Oldfield, E.H.: Magnetic resonance imaging of tumors and arteriovenous malformations of the spinal cord. Radiology 156:689-697, 1985.



11. Di Chiro, G., Knop, R.H., Girton, M.E., Dwyer, A.J., Doppman, J. L., Patronas, N.J., Gansow, O. A., Brechbiel, M.W., and Brooks, R.A.: MR cisternography and myelography with gadolinium-DTPA in monkeys. Radiology 157:373-377, 1985.
12. Di Chiro, G., Brooks, R.A., Girton, M.E., Caporale, T., Wright, D.C.: Studies of intracerebral hematomas in monkeys. AJNR 7:193-199, 1986.
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16. Giovanni Di Chiro.: MRI Contrast Agents for CSF Cavities. In Contrast Agents in Magnetic Resonance Imaging. James, A.E. and Felix, R. (Eds.). Excerpta Medica. In press.
17. Di Chiro, G., Girton, M.E., Frank, J.A., Dietz, M.J., Gansow, O.A., Wright, D.C., Dwyer, A.J.: Depiction of cerebrospinal fluid rhinorrhea in dogs by MR cisternography. Radiology. In press.







# ANNUAL REPORT

October 1, 1985 - September 30, 1986

## Instrumentation and Computers Section

National Institute of Neurological and Communicative Disorders and Stroke

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## INSTRUMENTATION & COMPUTERS SECTION

National Institute of Neurological and Communicative Disorders & Stroke

October 1, 1985 - September 30, 1986

Bruce M. Smith, Ph.D., Chief

The Instrumentation and Computers Section provides technical support for investigators of NIMH and NINCDS IRPs by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; and (3) designing, specifying and managing laboratory computer systems for data acquisition and processing.

Additional services provided by the Section include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Section is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Section, he first meets with the Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether ICS (Instrumentation and Computers Section) will take on the project. If a commercially produced instrument will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, ICS will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Section Chief or the Assistant to the Chief agrees to accept a project, the investigator submits a standard work request form (available from ICS), signed by his Lab Chief. This form will state the nature of the instrument or service requested, and should contain as many details and specifications as the investigator can provide.

The project is then assigned to an engineer or computer staff member, who will confer with the investigator to formulate a set of specifications and a timetable and cost estimate for the project. ICS does not charge for services, but the investigator will be billed for the cost of the components used. Upon completion of the project, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Section's CAN.

### INSTRUMENTATION

The Section has a staff of five engineers and four technicians to design, develop, and fabricate electronic and mechanical instruments. The major effort is in the production of electronic instruments for basic neurophysiological research, and for clinical studies involving affective disorders. The following are brief descriptions of representative projects, chosen from a total of 278 projects undertaken this year.

(1) Patient Activity Monitoring System. The Section has continued to develop the Patient Activity Monitor (PAM) and the support hardware and software which forms the system.

(a) Monitor. The current version of the PAM has a memory capacity of 1024 locations and is in its fourth year of production. Fabrication, testing, and calibration of a set of 30 units begun last year has been completed and another set of 50 monitors is now in the initial phase of fabrication. Most of the older versions of the PAM have been retired. Approximately 100 monitors are in use, with the Section providing battery changes and repairs as needed. The injection-molded plastic case developed for the monitor is now being produced with an ABS plastic that contains minute stainless steel fibers. The integral mesh of these fibers appears to provide sufficient electrical shielding to prevent interference from static electricity. A nickel-based conductive paint had been used to solve the static problem but required a separate painting step and caused skin irritation in some patients.

(b) Computer Support. A new PAM computer interface, which uses the RS-232-C serial data format, has been developed. Based on a CMOS 8-bit microprocessor, this interface allows an inexpensive personal computer with a serial port to serve as a readout device. ICS is developing a PAM program for the Apple Macintosh personal computer to support this readout method. This program already provides most of the capabilities of at least 15 PDP-11 programs and is easier to use, maintain, and update. The current version of this program is being tested with the new interface in four laboratories. Four additional interfaces have been fabricated in support of collaborative efforts with groups outside the IRP.

(2) Microprocessor-based Rotometer. A third generation animal rotation monitor was completed that uses an 8-bit microprocessor to determine the clockwise and counter-clockwise rotations of one to four rodents in cylindrical cages and to hold this data for input into the serial port of a Macintosh computer. Software has been written which uses the capabilities of the Macintosh to store the data on disk, and to display the data in real-time histogram form. Powerful statistical programs now available allow complete data analysis directly on the Macintosh. Using this same design approach, a 16-channel rotometer is currently being developed. The combination of the Macintosh and the high-speed microprocessor system reduces the number of integrated circuits in the design by a factor of 20 over previous ICS 16-channel designs.

(3) EMG Data Acquisition System. A three-unit data acquisition system has been completed for use with ongoing EMG studies. The first unit conditions eight analog signals from mechanical transducers (e.g., torque motor position outputs). Each channel consists of an instrumentation amplifier, gain control, switch selectable filter control and DC offset adjustment. The second unit processes eight EMG signals by providing full-wave rectification, low-pass filtering, and gain and offset adjustments. The third unit interfaces the other two to a PDP-11 computer by providing 16 channels of buffers and sample/hold amplifiers. Additionally, I/O signals from the computer interface boards are brought out on the front and rear panels for use when needed. Extra computer I/O connectors were added and card slots left open to accommodate future expansion.

(4) EMG/EEG Matrix Interface. A distribution and buffering system is being designed that will individually route fifty neurophysiological signals to any one or more of 110 destinations consisting of A/D converters, strip chart recorders, audio amplifiers, log and antilog amplifiers, and special monitors. To accommodate this extremely large switching network, a programmable pin board with all inputs and outputs buffered is being developed in a standard 8 1/2 X 19 inch rack panel with attached card cage. Custom printed circuit cards have been designed to handle the large number of buffers, and ribbon cabling will be utilized for input/output and matrix block connections.



(5) Data Acquisition Preamplifier System. A four-channel amplifier system was designed to interface between low-level spectrophotometer analog outputs and an A/D converter connected to a personal computer via a serial port. The gain for each amplifier channel is continuously variable between 0.5-1000. A dual-purpose offset adjustment was provided that simultaneously cancelled both input and output offset errors in the final amplifier stage, thus eliminating several active components. The system was mounted on top of the A/D converter in a closely-matched plastic cabinet.

(6) Photoglottography Detector. Accurate measurement of larynx activity during speech therapy studies using a bifurcated fiber optic bundle attached to a video camera has been limited by the 60 frames/second video recording rate. Fundamental frequencies of the vocal chords can be as high as 400Hz. In addition, it is desirable to monitor the time course of the opening width of the vocal chords. A self-adhering photodiode transducer was developed that measures both the frequency content and the quantity of light passing through the vocal chords by sensing the light leakage that escapes through the skin just below the Adam's Apple. The device is currently undergoing clinical evaluation.

(7) Isolated Linear Amplifier. A low distortion DC-25kHz optically-coupled amplifier has been developed to generate high-voltage isolated stimuli pulses for cell studies. A feedback type optically-coupled amplifier was utilized for the low distortion isolation and a high-voltage, high-slew rate operational amplifier with switchable gain ranges was used for the final stage. Output characteristics were 20  $\mu$ sec pulses at  $\pm$  20 volts peak into a 5k $\Omega$  load.

(8) Constant Current Stimulator. This device presents a bipolar current pulse through concentric electrodes placed on a subject's forearm as part of stimulus threshold studies. Current pulses from 1 to 31mA are controlled in one milliamp steps by a 5-bit digital word from a PDP-11 computer or manually by binary-weighted toggle switches. A trigger pulse from the computer or a pushbutton generates a bi-phasic stimulus of 2msec. duration. The stimulator design employs a center-tapped 100:1 transformer output stage supplied on the primary by a high-current DC supply in order to provide symmetrical pulses up to 31mA into load impedances equal to or less than 50k $\Omega$ .

(9) Dual Electrode Stimulator. This device allows two sets of electrodes to be used for both stimulation of rat brain tissue and for recording from the same sites. The two pairs of electrodes are switched from a strip-chart recorder to two constant current stimulators; after stimulation, the electrodes are briefly depolarized and then switched back to the recorder. This system is a dual version of units previously built by ICS and has been updated to include new technologies. The unit is equipped with logic to sample the stimulus current pulse, hold this value and display it on a panel meter. The display feature allows the user to monitor the stimulus pulses during an experiment.

(10) Attention Monitor. A simple microprocessor-based device has been designed to present a challenge test to subjects while they are exposed to a two-hour light stimulation during normal sleeping hours. On the average of once a minute, a four-second visual cue is presented. If the subject responds while the cue is on, a "hit" is recorded; no response results in a "miss" and a short alarm is sounded to regain the subject's attention. After the two-hour session ends, the time course of responses is printed so that the subject's attention pattern can be used in the evaluation of the efficacy of the light stimulation.

(11) Pulse Generator System. A multi-channel timing instrument (pulse generator system) is a vital part of many neurophysiological experiments. Instruments used within the IRP that were purchased about 17 years ago are no longer manufactured and have become unreliable. Newer, commercially available units lack the flexibility and convenience of the older devices. Two years ago, ICS designed a five-channel pulse generator system to fill this void. Six of these instruments were fabricated last year and an additional six units were completed this year.

(12) Neurophysiological Data Preprocessor. A microprocessor system was developed to replace the custom logic circuitry presently used by the Laboratory of Neurophysiology Data Acquisition System. This preprocessor records the times of occurrences of 64 different events and eight different pulses. This information is transmitted to the main processor (a PDP-11 minicomputer) through a parallel interface and the information is coded in such a form as to ensure compatibility with existing software that is used for analysis and display of the data. The preprocessor decreases response time to events and pulses and it frees the main processor for experiment control. Last year ICS constructed six units for the LNP/NIMH and two units for the LNLC/NINCDS. A user's manual for this system is now being developed and a search is being conducted for a private manufacturer to produce and market this system.

## COMPUTERS

Small computers are ideally suited for laboratory research in neurophysiology and psychology. They are used in the laboratory for on-line, real-time interactions, process control, and data acquisition. Recorded data may be stored, combined with other data, reduced statistically, transferred to larger computers for further analysis, transformed for presentation graphically or mathematically, and the results may be printed or plotted. Increasing use is being made of the small computer for processing the text of scientific papers and communications. Data base management is now available for the small computer, as are limited management information systems.

Techniques have been developed for image processing which are applicable to many diverse experimental systems, ranging from autoradiographs of brain tissue sections to the analysis of two-dimensional electrophoresis gels.

Larger minicomputers, the so-called super-mini's, have been reduced in price and are now available for functions formerly performed by larger time-shared systems. These systems allow applications in modeling, curve fitting and statistical treatment that would be prohibitively expensive on large systems.

Inexpensive personal computers are proving useful for dedicated applications. Many scientists are developing software for these computers, which they offer to the scientific community at low cost. PCs will become increasingly useful in the laboratory and their potential should be exploited.

Microcomputers incorporated in the design of biomedical instrumentation provide a savings in design and fabrication time for instruments, and a more flexible system than one based on discrete components.

The Instrumentation and Computers Section is actively involved in the applications of small computers in the IRP. By integrating the functions of biomedical instrument design and laboratory computer systems with software designed specifically for the research community, the Section offers computer support services for a broad range of scientific disciplines.

## LABORATORY COMPUTERS

The design goal for the laboratory instrument computer is to provide maximum function, tailored to the specific experimental design, with minimum cost. ICS provides consultation on the specification and selection of laboratory computers for new applications; conducts systems studies in collaboration with the scientist; and helps the scientist in the procurement, installation and maintenance of the equipment. In support of these efforts, ICS maintains a PDP-11/73 central computer in Bldg. 36 for program development and training. Additionally, a multi-user VAX-11/750 managed by ICS in Bldg. 36 provides high-capacity data storage, and efficient data processing, including graphic functions with plotting and printing on a high-resolution laser printer.

ICS provides training for the scientist or support personnel who will be programming and maintaining the laboratory computer system. Personnel limitations make it difficult for ICS to provide complete programming for specific individual applications, so such programming must be supplied by the laboratory. ICS computer personnel are always available for consultation, training, and help in debugging, as well as assistance in the selection of part-time programmers or consultants. Commercial software packages or applications from other research labs are often available, and ICS will evaluate such systems.

ICS develops and maintains a library of procedures which are written specifically for the laboratory computers used in the intramural community. These procedures are designed to be incorporated into the users' programs. In addition, ICS will aid the investigator in writing the difficult time and data dependent sections of real-time programs. ICS also develops some application programs which will have wide use within one or more laboratories or will support data acquisition hardware developed by ICS.

There are now more than 60 minicomputers in the program; many of these systems have been in use for years. A significant number of library procedures and general-purpose application programs are used on these machines. As experimental protocols develop and change, software changes are often required, so program maintenance is a continual and time-consuming function of the Section. This effort is aided by structured programming techniques and standardization of laboratory computers and peripheral equipment.

## VAX COMPUTER SYSTEM

ICS manages a multi-user VAX-11/750 computer system that is available to all IRP investigators. The VAX is located in Building 36, in space furnished by the Laboratory of Cerebral Metabolism, NIMH. Users in the building can have hard-wired cable connections installed for high-speed communication with the VAX, or can access the computer on a dial-up basis. In striving to improve the VAX as a useful research tool, ICS has installed a variety of new software, described below.

To aid research in molecular biology, the Section has assembled a system for convenient entry, analysis, and publication of nucleic acid and protein sequences. This comprehensive set of software and sequence databases provides IRP investigators with a no-fee alternative to a similar system on the DECSys-10. An important part of the system are Pearson's FASTN and FASTP programs which enable an investigator to quickly determine whether a new sequence is homologous to any entries in the latest GenBank™ nucleic acid database or NBRF protein database that are maintained on the VAX. Also available are Staden's DBSystem for management of large shotgun sequencing projects, Staden's AnalySeq and Diagon programs for analysis of

newly-acquired sequences, and NBRF's NAQ, PSQ, ALIGN, and PRPLOT programs.

For graphics support, ICS has developed PlotLib, a device-independent graphics package, which generates graphs on numerous display terminals and hardcopy devices. PlotLib-based software and a high-resolution Talaris laser printer permit publication-quality graphs and documents to be quickly and easily produced with a variety of programs. Printed documents may incorporate superscripts, subscripts, and Greek letters.

DATAPLOT, an interactive program featuring a high-level language for curve fitting and graphics, is now installed on the VAX. Similar to MLAB on DCRT's DECSys-10 computer, DATAPLOT is a tool for experimenting with mathematical models, as well as summarizing and analyzing data. Publication-quality graphs produced by DATAPLOT can be plotted on the Talaris laser printer.

An Ethernet local area network is being installed on the second floor of Building 36. It will allow experimental data acquired by laboratory PDP-11s to be quickly transferred to the VAX for analysis and plotting. This network can be extended to other floors in Building 36 if the initial installation on the second floor is successful.

### IMAGE PROCESSING

ICS maintains a general purpose image processing system consisting of an Optronics rotating drum film scanner, a Gould/DeAnza image array processor, and a PDP-11/34 computer. Images to be processed may be obtained by scanning autoradiographs, x-ray film, or photographic negatives, or by using images generated by CAT or ECAT scanners. A camera station is available to generate color hardcopy using Polaroid SX-70 or 35mm film.

Software packages that are easy to learn and use have been developed to provide an extensive and expandable repertoire of basic image processing functions. Special purpose functions can be developed to meet specific user requirements. The facility is useful for numerous applications involving evaluation and quantification of biomedical images. The two primary applications of the system are the densitometric analysis of autoradiographs of brain or tissue sections and the analysis of two-dimensional electrophoresis gels.

ICS has developed a new PDP-11/73 based image processing system that is capable of using these software packages. This system uses a TV camera for digitizing images instead of the rotating drum film scanner. Unlike the drum scanner, which can only digitize transparencies, the TV digitizer permits any object that can be placed under a camera to be digitized. This system will have a high-speed Ethernet link to the VAX-11/750.

### PERSONAL COMPUTERS

ICS has evaluated Apple Macintosh personal computers for potential use in both scientific and administrative applications. The Macintosh was chosen for its ease of learning and use, advanced design, and high quality graphics. It has proven useful in many areas, such as scientific word processing, where its ability to easily produce text containing equations, Greek letters, superscripts, and subscripts make it a very cost-effective alternative to expensive and inflexible dedicated word processors. The Macintosh has also reduced both the cost and time involved in making posters, slides, and publication-quality charts and tables. When used with the Apple Laserwriter printer, print quality is as good as, or better than, that of a dedicated word processor.

The Macintosh has also proven to be an effective graphics workstation for use with the VAX, DECSystem-10, Wylbur, and MEDLINE. An inexpensive program called VersaTerm allows the Macintosh to function as either a VT-100 compatible, full-screen editing terminal or as a Tektronix 4014 compatible graphics display terminal. VersaTerm allows text and graphics generated by the VAX to be printed on any Macintosh printer and it supports file transfer between host computers and Macintoshes.

In order to help IRP scientists with their scientific word processing and graphic workstation tasks, ICS maintains two Macintosh personal computers connected to an Apple LaserWriter printer in the central computing facility in Bldg. 36. ICS selects new Macintosh programs for statistical analysis, plotting, drafting, word processing, etc., and makes them available for evaluation at this facility.

The Macintosh is being used in four ICS projects for low-speed laboratory data acquisition and control. The first project involves presenting stimuli (various words or geometric designs) to Alzheimer's patients with recording of patient responses. A second project uses the Macintosh to control and collect data from an HP 8450 Spectrophotometer. The third project uses the Macintosh to log data generated by a four-channel rodent rotometer developed by ICS. The fourth project uses the Macintosh as a readout station for the Patient Activity Monitors supported by ICS.

While the main emphasis in personal computers by ICS staff has been on the Macintosh system, a smaller effort is under way to develop expertise with the IBM personal computer. One staff member has become involved in the DCRT Personal Workstation Office lead user program and continues to gain expertise through advanced DCRT courses. ICS is developing an IBM-AT PC system as an engineering workstation and as a prototype for laboratory data acquisition projects.

## MICROPROCESSORS

High-speed 8- and 16-bit microprocessors have become important design tools for the development of biomedical instrumentation by ICS engineering staff. Current CMOS low-power versions of these chips allow the design of both smaller, more reliable bench instruments and more intelligent portable devices. ICS maintains a complete Intel system for development of projects based on the 8085 and 8088 family of chips. Software development tools running on the Macintosh are available for support of Motorola/Hitachi 6801 and 6805 single-chip processors.

## ENGINEERING, COMPUTER, AND FABRICATION SERVICES

This table shows the distribution of the Section's workload among the various laboratories and branches. We have listed only the major users.

<u>LABORATORY OR BRANCH</u>	<u>PERCENT</u>
Medical Neurology, NINCDS	13.68
Neurophysiology, NINCDS	12.79
Clinical Psychobiology, NIMH	11.67
Biological Psychiatry, NIMH	8.57
Cerebral Metabolism, NIMH	4.65
Experimental Therapeutics, NINCDS	4.62
Biophysics, NINCDS	4.24
Child Psychiatry, NIMH	3.46
Clinical Science, NIMH	2.86
Neuropsychology, NIMH	2.62
Clinical Neuroscience, NIMH	2.45
Cell Biology, NIMH	2.45
Neurophysiology, NIMH	2.38
Neurochemistry, NINCDS	2.25
Neurobiology, NINCDS	1.65
Molecular Biology, NIMH	1.43
Neurochemistry, NIMH	1.41
Molecular Genetics, NINCDS	1.16
Neural Control, NINCDS	0.84
Neuropsychiatry, NIMH	0.81
Surgical Neurology, NINCDS	0.74
Central Nervous System Studies, NINCDS	0.53
Experimental Neuropathology, NINCDS	0.51
Psychology & Psychopathology, NIMH	0.34
Neuropathology & Neuroanatomical Sciences, NINCDS	0.31
 *NIMH (TOTAL)	 45.74
 *NINCDS (TOTAL)	 43.74
 *NICHD (TOTAL) **	 8.22
 *NIAAA (TOTAL)***	 <u>2.30</u>
	100.00

\*These figures represent our total effort; they include labs not listed individually.

\*\*This figure represents our support of NICHD which loans the Section one FTE.

\*\*\*This figure represents our support of collaborative efforts between NIAAA and NIMH.







# ANNUAL REPORT

October 1, 1985 through September 30, 1986

## Laboratory of Biophysics

National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report  
October 1, 1985 thru September 30, 1986  
National Institute of Neurological and Communicative  
Disorders and Stroke  
Laboratory of Biophysics  
William J. Adelman, Jr., PhD, Chief

## INTRODUCTION

Research in the Laboratory of Biophysics (LB) is concerned with achieving an understanding of the molecular basis for the functioning of neuronal cells, tissues and systems. The laboratory has two units. The Woods Hole (WH), Massachusetts unit has two sections: Neural Membranes (NM) and Neural Systems (NS), both located at the Marine Biological Laboratory. The Bethesda unit is the Section on Molecular Biophysics, located in Bldg. 36 at NIH. 1986 marks the eleventh year of continuous operation of the Woods Hole unit of LB.

LB has long been a leader in the study of membrane ion channels. This study has developed concepts of channel structure and function which have provided an understanding of the mechanisms for generating nerve impulses, synaptic activity, and higher integrative behavior of nervous systems.

Biophysical methods are coupled with modern ultrastructural and biochemical techniques in order to investigate complicated neuronal mechanisms at fundamental levels. These interrelations are not strictly conceptual, as methods, techniques, equipment and personnel develop in parallel and become part of the force and direction of LB. There are close ties in this connection between the Woods Hole and Bethesda units of LB.

### Section on Neural Membranes.

The Section on Neural Membranes uses electrical, chemical, optical, electron optical, mathematical and computer science techniques to investigate the function of neural cells and tissues at limits approaching molecular levels. Thus, molecular structures responsible for membrane ionic channel function and axoplasmic transport are sought. Model systems are constructed, tested and developed to simulate a variety of neuronal functions.

The Section has completed the second and third phases of its study of the sodium channel gating mechanism in the squid axon. Gating current records were obtained from internally perfused squid giant axons with intact sodium inactivation in sinusoidally driven dynamic steady-states and as dynamic transients as functions of the mean membrane potential and the frequency of the command sinusoid. Records were obtained before and after internal protease treatment of the axons which fully removed inactivation. The non-linear analysis consisted of determining and interpreting the harmonic content in the current records. The results indicate the presence of three kinetic processes, two of which are associated with activation gating (the so-called primary and secondary processes), and the third with inactivation gating. The dynamic steady-state data show that inactivation gating does not contribute a component to the gating current, and has no direct voltage-dependence of its own. Rather, the inactivation kinetics appear to be coupled to the primary activation kinetics, and the coupling mechanism appears to be one of reciprocal steric hindrance between two

molecular components. The mechanism allows the channel to become inactivated without first entering the conducting state, and will do so in about 40 percent of depolarizing voltage-clamp steps to 0 millivolts. The derived model kinetics further indicate that the conducting state may flicker between open and closed with the lifetime of either state being 10 microseconds. Dynamic transients generated by the model kinetics (i.e., the behavior of the harmonic components as a function of time following an instantaneous change in the mean membrane potential from a holding potential of -80 mV) match the experimental dynamic transients in all details. These transients have a duration of 7 to 10 milliseconds (depending on the level of depolarization), and are the result of the developing inactivation following the discontinuous voltage change. A detailed hypothetical molecular model of the channel and gating machinery has been constructed. A new mathematical model of the sodium channel gating kinetics has been formulated and programmed with appropriate algorithms to run on several different computer systems.

The sodium channel primary amino acid residue sequence suggests that the processes occur at four molecular locations in parallel. At present, experiments using chemical agents which specifically screen the charges on lysines and arginines are being performed to test the inferences drawn from model predictions. As expected, both arginine and lysine blockers profoundly alter channel gating kinetics in predictable ways.

The project comparing ionic current channels in nerve and heart cell membranes has elucidated the role of potassium channel currents in spontaneous activity of excitable membranes. Of particular significance has been the finding that derivatives of triethylammonium ions block potassium channels according to their molecular size. Small blockers, such as methyltriethylammonium and tetraethylammonium block without altering channel gating; large blockers such as n-pentyltriethylammonium and n-nonyltriethylammonium alter channel gatings as well. Moreover, potency of blockade increases with size of the blocker. These results are relevant to the mechanism of antifibrillatory drug action in the heart. In particular, potassium channel blockade appears to be the critical factor in preventing fibrillation. For example, a potent blocker of potassium current, such as n-nonyltriethylammonium, produces a significant antifibrillation effect, whereas a relatively ineffective blocker of potassium current, such as methyltriethylammonium produces relatively little antifibrillatory effect. The mechanism of antifibrillatory action is related to current flow between cells in the heart. By impeding the flow of potassium ions, antifibrillatory compounds change conduction pathways in the heart. Instead of running across individual cell membranes, current now flows more readily between cells. The process synchronizes the beating of heart cells and stops fibrillation, as the cells again contract in unison. The squid axon has played a significant role in this work by providing a rapid and effective screening technique for testing the potency of potassium channel blockade of putative antifibrillatory compounds.

The effort by the Section in improving image processing techniques continues in both electron and light microscopy. Three-dimensional electron microscopy of thick sections using successive specimen tilts and Fourier processing of images was advanced by the design, manufacture, and installation of a new EM column screen for digital video visualization of thick sections. Making use of LB's computer based image processing system, both light and electron microscopic images have been analyzed.

Work on primary culture of squid neurons has been highly successful and these cultured neurons are now used routinely for biophysical and physiological studies by LB and others. These cells have been identified as neurons by immunofluorescent assay with tetanus toxin. Somal diameters range from 5 to 40  $\mu\text{m}$  and neurite processes are several mm long. Embryonic cells from two other molluscs, Hermissenda and Octopus, also grow under the same conditions. Neurite growth has been observed using the image processing system described above.

Squid cultured cells (and several mammalian cell lines) send out filopodia as they attach to the substrate. The slower growing squid cells facilitate the study of filopodia motility. A "slip-knot" action is present which has not been described previously. This manifestation of motility appears to be a new fundamental dynamic property of cells. These studies are now providing new insights on the development of neurons and on neuronal connectivity.

The squid giant synapse has proved useful in providing a data base for developing a new hypothesis on exocytosis. Making use of observations of the role of Ca ion in transmitter release, the model proposes that the entry of Ca leads to K channel activation and K accumulation in the vesicles. Both anion and water movements follow leading to vesicle expansion, fusion with the membrane and eventual release. This model is now under test and preliminary results have identified both a Ca-activated K permeable channel and an anion channel in secretory vesicles.

Overall, the program of the Section has been able to reach very general realizations as to basic neural mechanisms. These generalizations have most often been the result of specific work on either the squid giant axon or the vesicles and membranes associated with the squid giant synapse. Future research plans involve, to a large extent, continuing making use of this fruitful preparation and extending to other systems the insights gained from the squid work.

### Section on Neural Systems.

The Section on Neural Systems takes a multidisciplinary approach to the question of how information is stored during associative learning and how it is made available for later recall. Biophysical and molecular mechanisms of associative learning are being analyzed in parallel for a mollusc (the sea snail, Hermissenda) and a mammal (the rabbit). Parallel analyses offer the important opportunity for uncovering general cellular principles of learning and memory - principles which have been conserved over the course of evolution and which, therefore, could have relevance for human cognition. Parallel analyses also permit exploitation of critical experimental advantages unique to these two species. For Hermissenda we have been able to demonstrate the first causal relationship of biophysical and molecular transformations within individual neurons to Pavlovian conditioning of a living animal. Causal relationships of cellular physiology and associative learning have not yet been approximated for any vertebrate preparation. Nevertheless, we have found evidence of biophysical transformations which are common to both mollusc and mammal. An identified group of neurons, the CA1 cells (rather than individual identified neurons) was shown to have a distribution of conditioning-specific modification of K channels within hippocampal slices removed from rabbits on days after they had been conditioned. Such slices provide vastly greater amounts of tissue (than does

the snail) for biochemical studies. Indeed, we have already observed conditioning-specific translocation of C-kinase, not only in the hippocampus CA1 neurons, but also in a restricted region of the cerebellar cortex called "H6". Furthermore, conditioning-specific changes of DNA metabolism closely followed the C-kinase translocation. C-kinase regulation of identical  $K^+$  channels occurs in both Hermissenda and hippocampal neurons. In Hermissenda, this regulation is being pursued with isolated membrane patches whose intracellular surfaces are accessible to precise ionic and biochemical manipulations.

The experimental psychology program of the Section uses associative learning paradigms to produce persistent behavioral changes in the nudibranch mollusc, Hermissenda crassicornis, as well as vertebrate species such as rabbits. Quantitative assessments are made of the animals' responses to the conditioned and unconditioned stimuli before and after classical conditioning paradigms. These assessments include precise dissection of generalized behavioral transformations into modification of individual muscular components of the behaviors. A full range of psychological manipulations have been used to clearly establish the sensitivity of the learning behavior to the exact temporal relationship of the stimuli which are associated during acquisition of the learning. Also of interest to the psychologists is the close linkage of the learning behavior to the specific stimuli associated and discriminative functions involving those stimuli not associated.

The Section's neurophysiology program is concerned first with the definition of those neural systems relevant to the learning capability. Multiple intracellular recordings from pre- and postsynaptic neurons have been employed within the visual, vestibular and chemosensory pathways of Hermissenda to establish a working knowledge of the critical neural systems and to describe how information flows in a stepwise fashion beginning with the sensory cells at the input, continuing through integrating cells, and finally to motor cells at the output. A similar approach is being taken with the rabbit hippocampus, and critical afferent and efferent pathways within this structure. Neurophysiological correlates are then obtained (again for both the mollusc and the rabbit) for conditioned (as well as a variety of control) animals. These neurophysiological correlates are recorded in intact animals, isolated nervous systems, and isolated neuronal membranes. Based on such correlates, electrophysiological sequences are constructed to trace the transformation of the information in electrical terms of the neural systems.

The Section's biophysics program measures persistent modification of specific ionic channels during and following the learning. In the past, a two-microelectrode voltage clamp was employed to characterize genetically specified membrane currents within identified neurons which were demonstrated to play a causal role in the acquisition and retention of associative learning. More recently, the patch-clamp technique has made it possible to analyze these currents on a single channel level. This technique has been used in both the cell-attached and "inside-out" configurations to determine which subcellular biochemical processes (e.g.,  $Ca^{2+}$ -dependent phosphorylation) are critical for regulating those ionic channels which change during learning. All of these biophysical approaches have also been applied to unequivocally demonstrate that it is in fact persistent modification of specific ionic channels which encode a learned association for later recall.

The biochemistry research effort of the Section seeks to uncover the molecular basis for the persistent ionic channel modifications shown to underlie associative learning (both in Hermisenda and the rabbit). A variety of biochemical and molecular biological methods are being brought to bear for this purpose. Microgel analysis of phosphorylation of individual neuronal proteins, for example, has revealed that  $\text{Ca}^{2+}$ -dependent phosphorylation of a specific low-molecular weight protein changes within certain neurons of conditioned animals but not those exposed to control paradigms. Exposure of neurons to prolonged depolarization, which simulates the integrated visual-vestibular network effects on identified neurons during conditioning, is also followed by long-lasting phosphorylation differences for particular low molecular weight proteins. Furthermore, a number of intracellular manipulations have provided support for the hypothesis that learning-induced modification of ionic channels involves  $\text{Ca}^{2+}$ -calmodulin-dependent and possible  $\text{Ca}^{2+}$  and lipid-dependent phosphorylation. Such manipulations include iontophoretic injection of  $\text{Ca}^{2+}$ -calmodulin-dependent protein kinase (Type II brain), inositol triphosphate, or phosphatase, or preincubation with C-kinase activators such as phorbol esters or OAG. Modern molecular biological techniques are also now making available for the Section's use monoclonal antibodies to enzymes (e.g., the Type II kinase) implicated in the learning process. Other antibodies (to phosphatase) may also prove helpful for our reconstruction of the biochemical and associated biophysical sequences which make biological records of memory possible.

The cellular anatomy aspect of the Section's programs contributes in several ways to the various levels of inquiry into the learning process already mentioned. Ultrastructural measurements of the cells and their synaptic interaction has provided further definition of the relevant neural systems. Activity-dependent uptake of radioactive labels within these systems has been monitored by autoradiographic methods. Morphometric techniques, together with serial sectioning and computerized reconstruction, may uncover structural manifestations of the biophysical and biochemical changes already shown for neurons within conditioned but not control animals. Differential absorption spectrophotometry allows intracellular localization of fluctuations of cytosolic  $\text{Ca}^{2+}$  as they occur during different phases of the learning process. Cytochemical identification of individual neurons has also implicated neurochemical means of amplifying the  $\text{Ca}^{2+}$ -dependent modulation of the channels during learning.

Finally, the developmental biologists within the Section have established laboratory strains of Hermisenda. Such strains permit assessment of how genetic and environmental factors may interact to determine individual differences in the ability of the animals to undergo associative learning.

Perhaps most important in all of these efforts is the accumulated evidence that a remarkable similarity exists between means of encoding learned associations in the snail and the rabbit. The same learning-induced reduction of well-characterized  $\text{K}^+$  channels has been found to provide such encoding in Hermisenda as it does within identified neurons of rabbit hippocampal slices. Similar regulation of these channels appears to occur at the molecular level for both the mollusc and the mammal. Such parallel mechanisms may ultimately provide the basis for clinical intervention and thereby the amelioration of pathologic symptomatology.

## Section on Molecular Biophysics

As part of our study of the basic properties of ionic channels, we have determined the basis for the anomalous open probabilities of sodium channels modified by batrachotoxin (BTX). We have previously analyzed membranes containing exactly two BTX-modified sodium channels, and found that the probabilities that 0, 1, or 2 channels are open did not follow a binomial distribution, indicating that the channels are not identical, are not independent, or are neither identical nor independent. In order to distinguish among these possibilities, we assumed that the two channels were independent, and then calculated the open probabilities for each channel as a function of voltage. We then compared these probabilities with the open probability for a number of one-channel membranes. The comparison showed that the differences between open probabilities of two channels in the same membrane were significantly larger than the differences between single channels in different membranes. The unreasonableness of this result led us to the conclusion that the assumption that the two channels are independent was incorrect. In this way, we concluded that the channels are not independent. Our results also allow us to determine that there is negative cooperativity between the channels; when one channel is open, the other channel is less likely to open.

Another study on basic channel properties showed that when sodium channels open, sodium gating current rises very rapidly, without a time delay. This is consistent with the standard view that the gating current represents the movement of channel charge associated with the opening process. Previous evidence indicating a time delay was in error because of measurement artifacts.

The projects described above address questions relating to basic channel properties. Most of our work this year has addressed questions relating to the role of ionic channels in normal and pathological physiological functions. The central question we addressed is how calcium influx into a secretory cell causes the secretion of neurotransmitters or hormones. We proposed a channel model designed to fit the salient experimental data regarding the dependence of secretion on calcium concentration, the speed of the response, and the effect of stimulus frequency. The model postulates that secretory vesicles contain both Ca-activated K channels and anion channels. When calcium enters the cell, it binds to sites on the Ca-activated K channels, allowing these channels to open. The opening of these channels and the presence of anion channels cause K and anions to enter the vesicles, thus increasing their osmotic pressure and causing an influx of water. For those vesicles situated very close to the cell plasma membrane, this could lead to fusion with the membrane and exocytosis of the vesicle contents.

In order to test the above channel model, we have determined the characteristics of channels from secretory vesicles of the bovine neurohypophysis by reconstituting them into lipid bilayers. We have found channels that are permeable to K ions, open with increasing calcium concentration and with increasing membrane potential, and have a single-channel conductance of about 200 pS - all in agreement with properties of Ca-activated K channels. An interesting difference between these channels and other Ca-activated K channels is that, in steady state, these channels open for only a narrow range of calcium concentrations - approximately 0.1 to 0.5  $\mu\text{M}$ . For higher or lower calcium concentration, the channels are almost always closed.



By reconstitution from the same secretory vesicles, we have also found an anion channel. This channel is permeable to Cl ions, has a single-channel conductance of about 20 pS, opens with a probability that is relatively independent of membrane potential, and is blocked by the anion-channel-blocking compound DIDS (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid). The finding that secretory vesicles contain both this anion channel and the Ca-activated K channel provides strong support for the channel model of secretion.

The surprising finding that Ca-activated K channels from these secretory vesicles tend to close when the calcium concentration is increased in the sub-micromolar range suggests a possible explanation for the inverse dependence of parathyroid hormone (PTH) secretion on calcium concentration: the closing of Ca-activated K channels in vesicles of the parathyroid gland when the internal calcium concentration increases. To test this explanation, we have patch-clamped bovine parathyroid cells and observed the properties of Ca-activated K channels present in the cell surface. The steady-state properties of these Ca-activated K channels were found to be very similar to those of the bovine neurohypophysis secretory vesicles, in agreement with the explanation suggested above. A more direct test of this explanation would involve determination of the properties of channels in parathyroid vesicles, rather than parathyroid plasma membrane. Even our somewhat indirect test is significant, however, since any channels in the vesicles would become incorporated into the plasma membrane during the process of exocytosis.

Another question we addressed is the cause of the pathological neural responses caused by increased oxygen tension. There are a number of reactive derivatives of molecular oxygen, such as the superoxide anion, hydrogen peroxide, and the hydroxyl free radical, and we plan to determine the effects of these derivatives. Our studies with hydrogen peroxide on the lobster neuromuscular junction have indicated a decrease in glutamate-mediated excitatory transmission. This decrease was shown to be caused by both presynaptic and postsynaptic mechanisms. In addition to decreasing excitatory transmission, hydrogen peroxide also decreases GABA-mediated inhibitory transmission by suppressing the presynaptic release of GABA. A further effect of hydrogen peroxide is a reduction of short-term synaptic facilitation. We plan to compare these effects with the effects of other reactive derivatives of oxygen and with the effects of oxygen itself.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02087-13 LB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Function and Structure of Ionic Channels: Ion Interactions and Gating Mechanisms.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI:                      W. J. Adelman, Jr.                      Chief                      LB, NINCDS		
COOPERATING UNITS (if any) University of Minnesota (J. Fohlmeister); Marine Biological Laboratory, Woods Hole, MA (C. Tyndale, R. Mueller, R. Waltz).		
LAB/BRANCH Laboratory of Biophysics, IRP		
SECTION Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.6	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             In this study, sodium channel molecular kinetic transitions are resolved by gating current harmonics. <u>Gating (asymmetry) currents</u> were obtained from <u>voltage-clamped squid giant axons</u> in sinusoidally driven <u>dynamic steady-states</u> with frequency and mean membrane potential as independent variables. <u>Harmonic content</u> of the records as a function of the independent variables shows three kinetic sub-processes (primary and secondary activation, and inactivation), and the number of states and values of rate constants in each sub-process. Protease-treated axons have a <u>secondary activation</u> gating process with two states corresponding to closed and open activation gates. The strongly voltage-dependent primary process has at least five kinetic substates which determine probability for transitions in the secondary kinetics. Flickering between open and closed states is a natural kinetic consequence. The harmonic content of records from axons untreated with protease show that inactivation gating can block three of five primary activation states, thereby substantially reducing gating current. <u>Inactivation and primary activation</u> appear to be coupled by <u>reciprocal steric hindrance</u>. Inactivation gating has no direct voltage dependence. The <u>sodium channel primary amino acid residue sequence</u> suggests that the processes occur at four molecular locations in parallel. At present, experiments using chemical agents which specifically screen the charges on lysines and argenines are being performed to test the inferences drawn from model predictions. As expected, both argenine and lysine blockers profoundly alter channel gating kinetics in predictable ways.           </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02092-13 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Subcellular and Extracellular Structure Associated with Nerve and Muscle.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. J. Adelman, Jr. Chief LB, NINCDS

Other: R. V. Rice IPA Fellow LB, NINCDS

COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA (A. Hodge, R. Waltz, C. Tyndale, R. Mueller); Carnegie-Mellon Univ. (R. Worthington); Case-Western Reserve Univ. (R. Lasek, S. Brady, M. Fahim)

LAB/BRANCH

Laboratory of Biophysics, IRP

SECTION

Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.9

PROFESSIONAL:

3.7

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to examine the subcellular and extracellular structure of nerve and muscle and relate such structure to function. Electron microscopy in TEM, STEM and analytical electron beam probe modes, such as EELS and EDAX, determination of proteins contributing to these structures and structural modeling are methods used in this study. The following structures are probed: 1) Neuroplasmic lattice, 2) neurofilaments, 3) microtubules, 4) axolemma, 5) glial cell membranes, and 6) myofilaments. Methods developed and used in this study are: 1) Stereoscopic imaging, 2) optical autocorrelation, 3) fast Fourier transformation (FFT) of STEM video images, and 4) STEM video image filtering and image enhancement using reverse Fourier transformation. Video imaged light microscopy is used to study living neurons in differential interference contrast. A new method was developed for direct visualization of particle velocity distribution. By viewing image pairs separated by an appropriate time interval in sequential recording of the subject, the positive or negative parallax arising from particle motion results in the binocular image of a particle being perceived as raised or lowered relative to an immobile background plane depending on its direction of movement. The degree of perceived elevation is proportional to particle speed. Using this method, measured particle movement during axoplasmic transport in squid axons ranged from 0.05 to 0.75  $\mu\text{m}/\text{sec}$ . The dynamic behavior of filopodia in a variety of cultured neuronal cell types while attaching to substrate have been analyzed. The configurational changes, particularly branching and sliding of branch points, have been and continue to be a focus of this study with particular emphasis on specific localization of fibrous proteins such as f-actin using fluorescently-labelled phalloidin.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02606-03 LB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Chemical Transmission at the Squid Giant Synapse.</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: E. F. Stanley Visiting Scientist LB, NINCDS  Others: W. J. Adelman, Jr. Chief LB, NINCDS G. Ehrenstein Research Physicist LB, NINCDS		
COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA (C. Tyndale).		
LAB/BRANCH <u>Laboratory of Biophysics, IRP</u>		
SECTION a) Section on Neural Membranes (located at MBL, Woods Hole, MA 02543) b) Section on Molecular Biophysics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.3	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           Observations on <u>transmitter release</u> at the <u>squid giant synapse</u> have been used as the basis for a <u>new hypothesis for exocytosis</u>, termed the "<u>ion channel model</u>". The key feature of this model is the presence of a <u>Ca-activated K channel</u> and an <u>anion channel</u> on the <u>secretory vesicle membrane</u>. According to the model, the entry of Ca into the cytoplasm activates the K channel, allowing K ions to enter the vesicle and anions follow down their electro-chemical gradient. The resulting accumulation of ions in the vesicle increases its osmotic pressure, leading to water entry, vesicle expansion and, finally, fusion of the vesicle with the cell membrane. This model can account for <u>key features of exocytosis</u> in synaptic transmission such as the fourth power dependence of secretion on external Ca, the very short latency between Ca entry and activation of the release mechanism, and the phenomenon of facilitation. Our first test of the ion channel model has been to search for the <u>postulated ion channels</u> in the <u>vesicle membrane</u> by incorporating purified secretory vesicles into artificial membranes. In support of the model, we have identified both a <u>Ca-activated K permeable channel</u> and an <u>anion channel associated with these secretory vesicles</u>.         </p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02607-03 LB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function of Tissue-Cultured Invertebrate Neurons.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI:           W. J. Adelman, Jr.                      Chief    LB, NINCDS  Other:       R. V. Rice                              IPA Fellow    LB, NINCDS		
COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA (J. Harrigan and R. Mueller); University of Hawaii (J. Arnold).		
LAB/BRANCH Laboratory of Biophysics, IRP		
SECTION Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.8	PROFESSIONAL: 0.7	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p>The aim of this project is to culture neurons in the laboratory for use in studies of axoplasmic structure and transport. These cultured neurons are also to be used in connection with voltage clamp and patch clamp experiments of ionic channels and their conductances and gating mechanisms. A number of fluorescent immuno-assays have established that neurons, glial, and muscle cells are routinely grown from squid embryos by our method. In collaboration with Alan Hodge, we have found that filopodia of cultured cells undergo a peculiar motility involving a "slip-knot" action. This motility is also present in filopodia of mammalian cell lines but is more easily studied with squid cells. Both high contrast DIC video microscopy and scanning electron microscopy are being utilized along with fluorescent probes.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02608-03 LB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Comparative Aspects of Ionic Conductances in Nerve and Heart Cell Membranes.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. R. Clay                      Physicist                      LB, NINCDS		
COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA (R. Mueller, C. Tyndale); McGill University (A. Shrier); University of Minnesota (M. Bacaner).		
LAB/BRANCH Laboratory of Biophysics, IRP		
SECTION Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.4	PROFESSIONAL: 1.3	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           This project is concerned with a comparative analysis of <u>ionic current channels</u> in <u>nerve and heart cell membranes</u> and the relationship of these channels to electrical activity, with a particular emphasis on <u>potassium ion channels</u> in both preparations and the effects of various <u>ionic blockers</u> on these channels. During the past year the primary experimental preparations which have been used are <u>squid giant axons</u>, <u>chick embryonic heart cells</u>, and <u>mongrel dog hearts</u>. The mechanisms by which ionic blockers and other agents alter potassium ion currents in the squid and chick heart cell preparations have been investigated with the <u>voltage clamp technique</u>. This work has focused recently on the derivatives of <u>triethylammonium ions</u>. A major finding has been the discovery of a relationship between the size of the ionic blocker and the mechanism of blockade. Specifically, the smaller sized members of this sequence, such as <u>methyltriethylammonium</u> and <u>tetraethylammonium</u> block channels without altering channel gating, whereas blockade by larger sized ions, such as <u>n-pentyltriethylammonium</u> and <u>n-nonyltriethylammonium</u> is accompanied by an alteration of gating. The relationship between potassium channel blockade and the mechanism of <u>anti-fibrillatory drugs</u> has been further investigated with the <u>open-chested dog heart preparation</u> using quaternary derivatives of lidocaine. In particular, QX314 and QX572 produce a significant increase in ventricular fibrillation threshold, which is well correlated with the blockade of potassium current in the squid and chick heart cell preparations which these agents produce. The relationship between ionic currents and spontaneous electrical activity in the heart has been further investigated with our ionic current model of <u>embryonic chick atrial cells</u>. Addition of <math>10^{-6}</math> M <u>tetrodotoxin</u> alters the shape of the action potential without altering beat rate. An analysis of our measurements of <u>sodium ion current</u>, <math>I_{Na}</math>, from single cells using the <u>suction pipette</u> technique before and after application of TTX, together with our computer model, illustrates the role of <math>I_{Na}</math> in spontaneous activity.         </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02151-12 LB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Information Processing in Simple Nervous Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.L. Alkon	Medical Officer	LB NINCDS
Others:	C. Collin	Visiting Fellow	LB NINCDS
	J. Disterhoft	IPA Fellow	LB NINCDS
	M. Kubota	Visiting Fellow	LB NINCDS
	A. Kuzirian	Staff Fellow	LB NINCDS
	S. Naito	Special Expert	LB NINCDS
	H. Rasmussen	IPA Fellow	LB NINCDS

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543 (J. Harrigan, I. Lederhendler, D. McPhie); Boston University Marine Program (C. Chen; D. Coulter); Medical Research Council, Canada (B. Bank); Deutsche Forschungs. (H.-P. Hopp)

LAB/BRANCH

Laboratory of Biophysics, IRP

SECTION

Section on Neural Systems (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

9.0

PROFESSIONAL:

8.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The principal objective of the program is to define molecular and biophysical mechanisms of learning and memory. Emphasis is placed on learning and memory which can be related to human cognition. Ultimate goals of such research are to arrive at clinically meaningful interventions on the one hand, and, to design and construct artificial intelligence which has advanced learning and memory capabilities. With human cognitive function as the principle frame of reference, the research focuses on associative processes (such as Pavlovian conditioning) rather than non-associative behavioral modifications (such as sensory adaptation, habituation, arousal and sensitization). The biological basis of learning and memory is of interest at several levels of complexity: behavioral phenomena, neuronal systems, neuronal membranes, and molecular transformations. To literally reconstruct the physiology involved (and then to model it in artificial contexts) it is necessary to use both "simple system" preparations such as the nudibranch mollusc Hermisenda crassicornis as well as "complex system" preparations such as rabbits. the molluscan work thus far has yielded the first unequivocal biological record of an associative memory. This record consists of persistent transformations of specific ionic channels. Because these records have been found within the membranes of identified single neurons it has proven possible to define biochemical pathways which regulate such long-term membrane modifications as well as to analyze how this biological memory record is expressed by the integrative functions of an entire neuronal system. The work on the vertebrate brain offers two essential opportunities. First, the generality of mechanisms determined for much less evolved species can be tested. Remarkably, the same ionic channel transformations have been shown in our program to record associative memory in the rabbit as were found in Hermisenda. Rabbit neural systems have also provided sufficient quantities of tissue so that conditioning-specific alterations of critical enzymatic (e.g., C-kinase) pathways which control membrane excitability have recently been demonstrated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02088-13 LB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Function and Structure of Membrane Ionic Channels		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: G. Ehrenstein  Others: K. Iwasa M. Jia N. Moran	Research Physicist  Senior Staff Fellow Visiting Associate Visiting Associate	LB NINCDS  LB NINCDS LB NINCDS LB NINCDS
COOPERATING UNITS (if any) Weed Science Laboratory - AEQI, Dept. of Agriculture, Beltsville, MD. (C. Baire and C. Mischke) University of Connecticut (R. Satter)		
LAB/BRANCH Laboratory of Biophysics, IRP		
SECTION Section on Molecular Biophysics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.7	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             The first type of experiment involves membranes containing 2 <u>sodium channels</u> modified by addition of <u>batrachotoxin</u>. We found that the probabilities that 0, 1, or 2 of these channels are open do not follow a binomial distribution, indicating that the channels are not independent, are not identical, or both. To distinguish among these possibilities, we assumed that the channels are independent, and tried to determine whether this assumption is consistent with all our data. We found that the assumption is not consistent with the open probabilities for 1-channel membranes, and therefore that the assumption is incorrect; the channels must be dependent. We further showed that the type of dependence is <u>anti-cooperative</u>; i.e. the opening of one channel reduces the probability that the other channel will be open.           </p> <p>             Another type of experiment involves channels in the cells of the plant <u>samanea saman</u>. We performed <u>patch clamp</u> experiments to determine that these cells do indeed contain channels and to identify the type of channel. We found evidence for <u>potassium channels</u>, suggesting a mechanism for the opening or closing of the leaflets of the plant in light or dark, respectively. Our data are consistent with the view that light opens potassium channels in cells in the <u>extensor</u> region of the <u>pulvinus</u>, and that this results in an influx of potassium together with anions and water, causing the extensor to swell and the leaflets to open.           </p>		



## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02091-13 LB

## PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mathematical Modeling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. FitzHugh Research Physicist LB NINCDS

Other: G. Ehrenstein Research Physicist LB NINCDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Biophysics, IRP

## SECTION

Section on Molecular Biophysics

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.2

## PROFESSIONAL:

0.1

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Work was completed on modeling the spread of the fertilization membrane over the surface of a spherical marine egg. The main result is that a model based on the opening of calcium channels in internal organelles by inositol trisphosphate, which, in turn, is released through enzymatic reactions, can account for the observed spreading of the fertilization membrane. The project has been terminated because of the retirement of the principal investigator. The model has been published, and future work relating to it will be incorporated into Project Z01 NS 02609 LB.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02218-11 LB						
PERIOD COVERED October 1, 1985 to September 30, 1986								
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Affect of Drugs on Voltage-Dependent Ionic Conductance in Membranes								
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: D. L. Gilbert</td> <td style="width: 33%;">Research Physiologist</td> <td style="width: 33%;">LB NINCDS</td> </tr> <tr> <td>Other: E. F. Stanley</td> <td>Visiting Scientist</td> <td>LB NINCDS</td> </tr> </table>			PI: D. L. Gilbert	Research Physiologist	LB NINCDS	Other: E. F. Stanley	Visiting Scientist	LB NINCDS
PI: D. L. Gilbert	Research Physiologist	LB NINCDS						
Other: E. F. Stanley	Visiting Scientist	LB NINCDS						
COOPERATING UNITS (if any)  Georgetown University, Washington, D.C. (C. Colton); NMRI, Bethesda, MD (J. Colton); CNRS, Marseille, France (L. Fagni).								
LAB/BRANCH Laboratory of Biophysics, IRP								
SECTION Section on Molecular Biophysics								
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892								
TOTAL MAN-YEARS: 1.8	PROFESSIONAL: 1.6	OTHER: 0.2						
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews								
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>Experiments have been performed on the effects of <u>hydrogen peroxide</u>, a reactive derivative of <u>molecular oxygen</u>, on <u>synaptic transmission</u>. Hydrogen peroxide effects a decrease in <u>glutamate-mediated excitatory transmission</u>, caused by both <u>presynaptic</u> and <u>postsynaptic</u> mechanisms. It also effects a decrease in <u>GABA-mediated inhibitory transmission</u>, caused by suppression of the presynaptic release of <u>GABA</u>. A further effect of hydrogen peroxide is a reduction of short-term <u>synaptic facilitation</u>. These effects are somewhat different from the experimental effects of molecular oxygen, itself. Other reactive derivatives will be examined in order to elucidate the overall effect of molecular oxygen.</p>								

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02317-09-LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Excitable Membranes and Ion Channels in Cultured Nerve and Muscle Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. H. Lecar

Research Physicist

LB NINCDS

COOPERATING UNITS (if any)

LN NINCDS; Tissue Transplantation Program Center, NMRI (S. Yeandle)

LAB/BRANCH

Laboratory of Biophysics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated because of the resignation of the principal investigator before the beginning of this fiscal year. A review paper relating to this work was recently published.

Publication:

Lecar, H. Gated ionic channels and the mechanism of excitability. Fed. Proc. 44: 2941-2943 (1985).

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02526-05 LB
PERIOD COVERED <u>October 1, 1985 through September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Gated Ionic Channels in Membranes</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <span>PI: R. E. Taylor</span> <span>Research Physiologist</span> <span>LB NINCDS</span> </div>		
COOPERATING UNITS (if any) Dept. of Physiology, UCLA, Los Angeles, CA (F. Bezanilla, C. Webb) Duke University, Durham, NC (J.R. Stimers)		
LAB/BRANCH Laboratory of Biophysics		
SECTION <u>Section on Molecular Biophysics</u>		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.2	PROFESSIONAL: 1.0	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <div style="margin-top: 10px;"> <p>When artifacts of series resistance are eliminated, <u>sodium gating current</u> rises very rapidly, without a time delay. This implies that the gating current represents the movement of charge on the <u>channel</u>, itself, that occurs as part of the opening process. Our measurements of potassium accumulation are consistent with the view that previous measurements of a relatively slow rising phase were artifactual.</p> <p>Addition of <u>ATP</u> to the cytoplasm of the squid giant axon affects the behavior of the <u>potassium current</u>. The conductance-voltage curve becomes steeper and the conductance-time curve becomes slower.</p> </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02609-03 LB						
PERIOD COVERED <u>October 1, 1985 through September 30, 1986</u>								
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Mechanism of Egg Activation Following Fertilization</u>								
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: G. Ehrenstein</td> <td style="width: 33%;">Research Physicist</td> <td style="width: 33%;">LB NINCDS</td> </tr> <tr> <td>Other: J. Russell</td> <td></td> <td>NICHD</td> </tr> </table>			PI: G. Ehrenstein	Research Physicist	LB NINCDS	Other: J. Russell		NICHD
PI: G. Ehrenstein	Research Physicist	LB NINCDS						
Other: J. Russell		NICHD						
COOPERATING UNITS (if any) <u>Emory University, Atlanta, GA (L. DeFelice)</u>								
LAB/BRANCH <u>Laboratory of Biophysics, IRP</u>								
SECTION <u>Section on Molecular Biophysics</u>								
INSTITUTE AND LOCATION <u>NINCDS, NIH, Bethesda, Maryland 20892</u>								
TOTAL MAN-YEARS: <div style="text-align: center;">0.3</div>	PROFESSIONAL: <div style="text-align: center;">0.2</div>	OTHER: <div style="text-align: center;">0.1</div>						
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews								
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p style="margin-left: 40px;">         We have previously demonstrated that a <u>fertilization membrane</u> forms around a <u>sea urchin egg</u> when it is injected with a soluble <u>spermatazoa</u> fraction <u>isosmotic</u> with seawater. Work is proceeding to purify the sperm extract. In addition, we have found that injection of a very small amount of distilled water will cause the formation of a fertilization membrane, presumably by <u>osmotic lysis</u> of an internal organelle and consequent release of its contents.       </p>								

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02709-01 LB															
PERIOD COVERED October 1, 1985 through September 30, 1986																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Secretion of Neurotransmitters and Hormones																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Scientific Personnel:</td> <td style="width: 33%;">PI: G. Ehrenstein</td> <td style="width: 33%;">Research Physicist</td> </tr> <tr> <td></td> <td>Others: E. F. Stanley</td> <td>Visiting Scientist</td> </tr> <tr> <td></td> <td>M. Jia</td> <td>Visiting Associate</td> </tr> <tr> <td></td> <td>S. Pocotte</td> <td>Staff Fellow</td> </tr> <tr> <td></td> <td>J. Russell</td> <td>NICHD</td> </tr> </table>			Scientific Personnel:	PI: G. Ehrenstein	Research Physicist		Others: E. F. Stanley	Visiting Scientist		M. Jia	Visiting Associate		S. Pocotte	Staff Fellow		J. Russell	NICHD
Scientific Personnel:	PI: G. Ehrenstein	Research Physicist															
	Others: E. F. Stanley	Visiting Scientist															
	M. Jia	Visiting Associate															
	S. Pocotte	Staff Fellow															
	J. Russell	NICHD															
COOPERATING UNITS (if any)																	
LAB/BRANCH Laboratory of Biophysics, IRP																	
SECTION Section on Molecular Biophysics																	
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																	
TOTAL MAN-YEARS: 2.6	PROFESSIONAL: 2.4	OTHER: 0.2															
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             We have performed several tests of a <u>channel model</u> for the role of <u>calcium in exocytosis</u> (Cf. Project Z01 NS 02606 LB). The model postulates that <u>secretory vesicles</u> contain both <u>Ca-activated K channels</u> and <u>anion channels</u>. When calcium enters the cell, it binds to sites on the <u>Ca-activated K channels</u>, allowing these channels to open. The opening of these channels and the presence of anion channels cause K and anions to enter the vesicles, thus increasing their <u>osmotic pressure</u> and causing an influx of water. For those vesicles situated very close to the cell plasma membrane, this could lead to fusion with the membrane and <u>exocytosis</u> of the vesicle contents.           </p> <p>             Evidence in support of the channel model was obtained by <u>reconstituting</u> <u>Ca-activated K channels</u> and <u>anion channels</u> from secretory vesicles of the bovine <u>neurohypophysis</u> into <u>lipid bilayers</u>. An interesting and somewhat surprising property of these <u>Ca-activated K channels</u> is that, in steady state, they open for only a narrow range of calcium concentrations - about 0.1-0.5 uM. The closing of these channels as the calcium concentration increases suggests a mechanism that could explain the inverse dependence of <u>parathyroid hormone</u> (PTH) secretion on calcium concentration. <u>Patch clamp</u> experiments on parathyroid cells are consistent with this mechanism: <u>Ca-activated K channels</u> close down as calcium concentration is increased in the submicromolar range.           </p>																	







ANNUAL REPORT

October 1, 1985 through September 30, 1986

Laboratory of Central Nervous System Studies

National Institute of Neurological and Communicative Disorders and Stroke

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We have defined the new field of transmissible and non-transmissible brain amyloidoses and the concept that brain aging and many degenerative diseases of brain are the result of interference with axonal transport and resulting stagnation, pooling and collapse of the cytoskeletal elements with ensuing post translational modification of the secondary structure of host cytoskeletal proteins to  $\beta$ -pleated sheet constructed fibrils of amyloid. We are also largely responsible for the hypotheses that self-replicating proteins with properties resembling "viruses" may be responsible for autocatalytic patterned degradation of host precursor proteins to amyloids encountered and that minerals, especially silicon, aluminum, and calcium, may be involved as nucleating agents for the patterned degradation or crystallization deposition of insoluble amyloid.

Slow Unconventional Viruses Causing  
Transmissible Brain Amyloidoses

Our laboratory has concentrated its main effort on elucidating the relationship between the proteins specific for kuru, Creutzfeldt-Jakob disease (CJD), and scrapie and their host-specified precursors. It is now evident that the 27-30 kilodalton (kDa) protein found in kuru, CJD, and scrapie is a protease cleavage product of a 35-37 kDa precursor, which is present in normal and in infected brain tissue, but modified in the latter into a less soluble protease-resistant form. Infectivity has not been definitively associated with the 35-37 kDa protein, but this protein and the protease-truncated 27-30 kDa "prion protein" (PrP 27-30) assemble in vitro into congophilic, birefringent rods resembling the scrapie associated fibrils (SAF) of Merz, but in so reassembling there is no restoration of infectivity.

It remains to be proved whether this normal host protein modified by scrapie virus infection is itself the infectious agent (an amyloid molecule, patterning autocatalytically the modification of host protein precursor into its infectious autocatalytic form), or, hidden in the infectious preparations of SAF, there still remains the possibility of a very small nucleic acid fragment contributing a more conventional virus-like factor to the replication process. No non-host nucleic acid has been demonstrated, even in highly infectious preparations, and the improbable conjecture that the entire infectious process is that of autocatalytic modification of a protein precursor and a nucleating or seeding event leading to a patterned crystallization in the form of amyloid fibers now appears likely. The host gene specifying the 35-37 kDa precursor protein has been fully sequenced.

Polyclonal and monoclonal antibodies prepared against synthetic polypeptides of the N-terminus of the amyloid of scrapie reveal varying distribution and patterns of the epitopes in normal and infected tissues. Such antibodies have shown reactivity to the scrapie-associated proteins and, to our surprise, to many purified proteins, including purified natural and synthetic human growth hormone. This has led to great difficulty in using the antibody technique to screen cDNA libraries. However, these antibodies have been valuable in developing a diagnostic test for CJD, kuru, and scrapie in that, when conjugated with gold particles, the antibodies to SAFs or to the synthetic polypeptide specifically label purified SAFs from kuru-, CJD- and scrapie-infected brains. Such SAFs are not obtainable from brains of other human neurodegenerative diseases, and thus this new immunological gold-

labeling technique has been used to identify serologically the SAFs in the two patients with frozen brain available of the four patients who developed CJD from injections of contaminated human growth hormone preparations.

These immunocytochemical and molecular biological studies on the scrapie/kuru/CJD-associated proteins and their normal precursors are largely aimed at preparing them in high purity and sufficient amounts for crystallographic study, and investigation at the organic chemical level, of the fine structural modification involved in the conversion of normal host-protein into amyloid fibers which appears to be the major pathogenic reaction of these diseases.

For over two decades we have carefully saved at  $<-70^{\circ}\text{C}$  frozen tissues from chimpanzees and other nonhuman primates affected with the human Creutzfeldt-Jakob disease and Gerstmann-Sträussler syndrome viruses, kuru virus, and scrapie, and the frozen tissues collected from cats, hamsters, guinea pigs and other animals susceptible to these viruses. These were collected and saved for eventual biochemical study when this would be possible. It is now possible to process these tissues for PrP 27-30 protein, for SAF's, and for the 35-37 kDa scrapie-specific protein and its precursor. As more sophisticated study of the structure of these proteins is possible, we hope to determine from this material the contribution of the host to these subacute spongiform encephalopathy viruses or slow unconventional viruses. This we are in a unique position to do, since it would take from two years to over a decade for other laboratories to obtain infected brain material from a number of different species each inoculated with the same strain of virus.

We already have indications that there are many strains of CJD viruses. Using these tissues it is possible to answer the critical question of the relative contributions of the host and the virus strain to the pathogenesis of the disorders and the molecular structure of the virus strains. Also, specific epitope antigenic analyses of the different strains is possible, both in comparing strains of different human origin, and comparing the same strain as it has been passed in different species. This sets the scene for a program involving several years of intensive molecular biology: DNA and amino acid sequencing, gene cloning, and monoclonal and polyclonal antibody epitope analyses of the disease-specific polypeptides. The results should further open the new field which we have defined: transmissible and non-transmissible brain amyloidoses.

#### Non-transmissible Brain Amyloidoses of Aging, Alzheimer's Disease and Other Dementias

Amino acid sequencing of the 4 kDa polypeptide subunit of the paired helical filaments of neurofibrillary tangles (NFTs), in the amyloid plaque cores, and of amorphous amyloid in congophilic angiopathy indicates that all three pathognomonic structures of the aging brain, Alzheimer's disease, Pick's disease, progressive supranuclear palsy, and late Down's syndrome are composed of identical 4 kDa subunits. This 4 kDa polypeptide subunit which easily associates into dimers, tetramers, octamers, and hexadecamers, is completely different from that found in the transmissible cerebral amyloidoses.

We are on a quest for the gene that encodes for the precursor of this amyloid by using a synthetic oligonucleotide probe. Since antibodies to the synthetic polypeptides react with many other proteins, the antibody technique of locating the gene has proved unsuccessful. We have therefore synthesized a DNA probe of 55 nucleotides, in order to use it for selecting the gene from total cellular DNA and from the cDNA libraries by hybridization. The sequence selected for this synthetic probe has been so chosen as to be unusual in the

cDNA library. A similar approach using smaller probes and less carefully selected regions has already failed in the hands of our German colleagues, and we are thus in a competitive race with other laboratories in trying to find this precursor.

Since histological and immunochemical studies indicate that the amyloid found in the non-transmissible cerebral amyloidoses resembles masses of stagnated or pooled cytoskeletal elements, we have surmised that a component of neurofilament, specifically the 200 kDa component, is the precursor for this amyloid. We have managed to clone the gene for the 200 kDa neurofilament protein and we are in the process of sequencing it. This has been difficult because the fragments from restriction enzyme cleavage are showing enormous redundancy, and protein of such size with such redundancy is very rarely encountered. However, the demonstration that this protein is mostly alpha-helical, and unlikely to yield beta-pleated sheets, even with post-transcriptional modification, has prompted us to investigate whether the associated entrapment, pooling, and stagnation of the microtubule-associated protein  $\tau$  (MAP-tau), may be the precursor we are seeking. We are, therefore, cloning and sequencing the gene that encodes for MAP-tau.

We hypothesize that interference with axonal flow is a common mechanism of neuronal damage, whether the primary cause is toxicity (mineral or other), deficiency, genetic metabolic defect, viral infection, or trauma, and that this interference with axonal transport causes pooling and stagnation of cytoskeletal elements, most frequently neurofilament. This has been the basic paradigm which we have used for understanding the varying neuronal damage and early appearance of neurofibrillary tangles of Alzheimer's type which occurs in the foci of high incidence parkinsonism dementia or pure amyotrophic lateral sclerosis in the Western Pacific. Such a mechanism could underlie all these disease processes. We have now demonstrated that the accumulated amyloid of the neurofibrillary tangles in Guamanian patients is the same as that of Alzheimer's disease and consists of a subunit protein of about 4 kDa similar, if not identical, in antigenic structure and amino acid composition to that of the subunit protein of the amyloid in Caucasian aged brains, whether the patients have senile dementia or not.

#### The Carbohydrate of Glycosylated Amyloid Subunits

In addition to this major thrust of work in our laboratory, which involves molecular biology, genetics, immunology, microbiology, and biochemistry, we are also collaborating with H. Kobata's Laboratory of Carbohydrate Chemistry in Tokyo in an investigation of the carbohydrate moiety on the subunit of amyloid in the 27-30 kDa protein of kuru/scrapie/CJD which is heavily glycosylated. Many of our antibodies are directed against the sugar component rather than against the amino acid polypeptide.

#### In Vitro Production of Amyloid Fibrils

We have been studying the re-coiling of polypeptide chains into different fine structural configurations which permit beta-pleated sheet stacking into fibrils resembling the amyloid fibrils of human pathology. Thus, using a beta-2-microglobulin we have succeeded in producing fibers that have the conophilic and green birefringent properties of amyloid fibrils and also the electron microscopic appearance of such fibers and even paired twisted fiber structures. More specifically, we have produced amyloid-like fibers from potential precursors to brain amyloids, such as the 200 kDa protein of neurofilament, and are now trying with MAP-tau. The 200 kDa

neurofilament protein assembles in vitro into amyloid like filaments which are both congophilic and green birefringent and in morphology resemble amyloid fibrils, and the amyloid-like properties increase on partial cross-linking with paraformaldehyde fixation.

The possibility that the fine structural changes of the aging or disease brain may be reproduced in vitro is obviously intriguing.

#### Neurofilament Pathology in Human Neurodegenerative Diseases

By using monoclonal antibodies directed against neurofilament polypeptides, we have demonstrated an aberrant accumulation of the phosphorylated form of the 200 kDa subunit within spinal neurons and pyramidal neurons in the hippocampus and cerebral cortex of patients with Alzheimer's disease, parkinsonism-dementia, ALS, and CJD. Neurites in the senile plaques are also stained. The immunocytochemical staining is particularly prominent in neurons bearing neurofibrillary tangles. Small amounts of congophilic amyloid can be identified in the zones corresponding to the neurons positive for the phosphorylated 200 kDa subunit. By contrast, areas having "ghost neurons" filled with congophilic amyloid are usually negative on immunostaining.

These observations suggest that there may be a relationship between the post-translational modification of neurofilament proteins, such as phosphorylation, and assembly and transport. These alterations appear to be one of the common pathogenic mechanisms in the evolution of various neurodegenerative disorders, especially those associated with neurofibrillary tangles.

#### Creutzfeldt-Jakob Disease and Human Growth Hormone

A further area of intense involvement of our laboratory has been in the problem of Creutzfeldt-Jakob disease developing in recipients of human growth hormone prepared from pooled autopsy pituitary glands by the NIH and other programs. At least three different batches of growth hormone have been contaminated, since one patient occurred in England, where none of the American products were used, and no batch of hormone was used by all three American patients, although two shared some batches. Incubation periods have ranged from 4 to 15 years, and in one case with frozen brain we have been able to demonstrate both the PrP 27-30 protein by our laboratory's developed ELISA test for it, and to demonstrate, with immune electron microscopy using gold bead associated antibody, that the SAF preparations were specifically antigenically related to kuru, scrapie, and CJD virus. Moreover, in an additional HGH patient with motor neurone signs who was suspected having CJD, failure to detect the protein provided strong evidence against the diagnosis of CJD, and verified the subsequent neuropathological examination showing ALS. Primates have been inoculated with more than 50 separate batches of HGH, but incubation periods are a year or more. Intense surveillance is under way of some 40,000 other young people who received the hormone injection, and one further probable case has been identified.

#### Toward a Biochemistry of Silicon and Aluminum

The metabolic adjustment to severe environmental deficiency of calcium and magnesium which is responsible for the deposition of calcium, aluminum, silicon, phosphorous and other minerals in brain cells in early life in the

high incidence foci of amyotrophic lateral sclerosis (ALS) and parkinsonism dementia (PD) and the early appearance of Alzheimer's neurofibrillary tangles (NFTs) in isolated populations in the Western Pacific (Guam, Japan, West New Guinea) was first suggested by epidemiological and ecological studies. Mineral analyses of environmental specimens of soil and water confirmed this hypothesis. Finally, electron probe X-ray analyses using both energy dispersive and wave length dispersive spectroscopy has demonstrated these long term deposits in the brains of Guamanian ALS and PD patients and in normal individuals exposed to the same environmental deficiencies who show the early appearance of NFTs. When these  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  deficiencies are removed by increased access to outside foodstuffs, changed water supply, and improved transportation and economy all three diseases (Alzheimer's NFTs, ALS and PD) have declined markedly in incidence or disappeared within a period of two or three decades.

This discovery of the primary cause of all three pathological processes in the Western Pacific isolates has led to animal experiments which further substantiate the hypotheses (see below) and stimulated a renewed interest in the role of mineral deposition in interfering with axonal transport. Even therapeutic and prophylactic clinical regimens are now suggested and some are under study.

Furthermore, the role of silicon and its polymers in altering the secondary structure of proteins through long series of hydrogen bonds is now under investigation. Silicon and aluminum compounds can interact strongly with phospholipids, lipids, carbohydrates and oligonucleotides as well as with polypeptides. Thus, mineral deposits of montmorillonite clays--calcium-aluminum-silicates--and hydroxyapatites can denature and alter protein fine structure and conceivably play an active role in degradation of host precursor proteins to amyloids.

The recent confirmation of older observations of silicon containing deposits in the center of purified insoluble amyloid plaque cores from Alzheimer's disease patients and in Alzheimer's NFTs has greatly stimulated interest in the possible role of these silicon and aluminum containing mineral deposits as nucleating agents or even as autocatalytic agents in the deposition or crystallization of such amyloid deposits. The work and thinking of this laboratory in these directions has had a major impact in determining the course of modern inquiry into aging and the degenerative amyloidoses of brain, including Alzheimer's disease.

#### Possible Role of Low Dietary Ca and Mg And a Neurotoxin in the Evolution of Motor Neuron Disease

Oral administration of low calcium and magnesium diet to young cynomolgus monkeys (*Macaca fascicularis*) for a period of 4 years has induced degenerative changes and variable degrees of intracellular calcium accumulation in the motor neurons of the spinal cord and brainstem, and in the giant Betz cells of the cerebral cortex. Supplementation with low dose aluminum and manganese chloride has resulted in a cellular accumulation of argentophilic material of neurofilament origin in different areas of the central nervous system. None of the animals, however, showed overt clinical symptoms despite these neuropathological changes.

Immunohistochemical staining, using monoclonal antibodies against neurofilaments, has revealed an aberrant accumulation of the phosphorylated form of the 200 kDa subunit protein within the perikarya of motor neurons in the spinal cord, mesencephalic component of trigeminal nucleus, zona compacta of substantia nigra, and of large pyramidal neurons in the cerebral cortex.

This abnormal accumulation was noted maximally in animals fed aluminum and only minimally in those fed a low  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  diet alone.

In addition to the neuronal pathology, axonal spheroids were seen both in the neuropil of the nuclear area and white matter. As in ALS, the lateral and anterior columns of the spinal cord, the spinocerebellar tracts and cortico-spinal tracts in the brain stem revealed axonal swellings, spheroid formation and focal axonal loss. Gliosis was conspicuously absent.

These observations support the hypothesis that low  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  levels interfere with axonal transport of the neurofilament subunits. This is further accentuated by the addition of aluminum. It is believed that the compact, relatively rigid molecules of phosphorylated 200 kDa neurofilament proteins accumulate in the neuronal soma leading to functional derangement and eventually to cytolysis.

This nonhuman primate model provides a means to understanding the pathogenic mechanism involved in the evolution of lesions in motor neuron disease.

#### Human Lentivirus (AIDS) Encephalopathy in Children

Our laboratory is also working on the problem of the primary encephalitis which characterizes almost all cases of childhood AIDS acquired congenitally from a human immunodeficiency virus (HIV) infected mother. The HIV mothers are giving rise to infected babies in 80% of their offspring, and some 80% of these infected offspring develop clinical AIDS. Most develop disease within one to two years after birth; a few are as delayed as four to five years of age. All, however, develop a primary encephalitis characterized by dysarthria, speech impairment, with eventual aphasia, and severe midline truncal ataxias and loss of developmental milestones.

We have demonstrated the virus in the brains of these infants by in situ DNA hybridization, by fluorescent antibody localization, and by electron microscopy. In histological studies we have demonstrated that there is a specific neuropathology with large, multinucleated macrophages in the brain, loaded with virus particles that are visible by electron microscopy. Similarly, there are brain cells which appear to be astrocytes, bulging with the human lentivirus particles. In neurons, these virus particles are rarely seen, and when seen are few in number. It was from the awareness of the primary human lentivirus encephalopathy of infants and children that a search was made in the brains of adults, and similar pathology was found in more than half of the fatal cases of AIDS.

It is only in the last two years, therefore, that clinicians have become increasingly aware that many adult AIDS patients show varying degrees of dementia which is not due to opportunistic infection with mycoplasma, mycobacterium, yeast, toxoplasma, cytomegalovirus, or herpes simplex virus.

The human lentivirus has thus become the major cause of encephalitic death among children and adults in the United States. In adults and more frequently in children, primary encephalitis from human lentivirus infection may occur without an immune deficiency syndrome. Thus, we are dealing with another example of transmissible virus infection resulting in a chronic dementia.

#### Search for an Animal Model of AIDS

Our laboratory first demonstrated active infection of chimpanzees with LAV and HTLV-III and with primary human tissues obtained from AIDS



patients. The animals become seropositive but do not develop clinical disease, and if there is any alteration in immune function, it is a transient lymphocytosis with moderate impairment of lymphocyte function, but not a helper-suppressor ratio change equivalent to that in human AIDS. The animals show no clinical disease three years after inoculation but they remain seropositive and viremic.

Such chimpanzees developing primary infection on inoculation with human brain tissue from AIDS patients provided the first demonstration of the live virus in the brain of AIDS patients. Since such infection has occurred even at high dilutions of suspensions of brain tissue from AIDS, the presumption is that the virus is in brain and in considerable quantity.

Many other species of nonhuman primates have been inoculated without obtaining disease, or primary infection, or antibody conversion; however, an occasional rhesus monkey has demonstrated a transient viremia and antibody response although none have developed clinical disease. Similarly, juvenile rhesus monkeys inoculated with these human viruses do not develop a fatal encephalopathy equivalent to that caused by HTLV-III. The human lentiviruses do not produce disease, even though they are very closely related to HTLV-III. Thus, we are without a good experimental model for vaccine evaluation in small animals or in nonhuman primates, and all that can be done at present is to test for the ability of vaccines to protect against primary infection.

Our laboratory first introduced the studies of the Icelandic visna & maedi sheep diseases in the United States at the NINDB Symposium on Slow Viruses in 1962, to which the Icelandic workers were invited as participants. We made the first isolations in the United States of the visna virus, later defined as the prototype lentivirus, from the brain of a sheep with Montana sheep disease. The maedi virus previously was thought to be causing only pulmonary involvement in Montana sheep disease. It is now known to be the same virus as visna, which may cause maedi, or zoegersiekte, in Iceland and the Netherlands, respectively, the pulmonary forms of visna virus infection.

AIDS virus (or HIV) belongs to this group, now called the lentiviruses. The entire group has been named a lentivirus sub-group of the Retroviridae, and the AIDS viruses are the first human representatives of the group, which contains visna virus, equine infectious anemia virus, and caprine arthritis encephalitis virus. We have found that horses inoculated with the human AIDS lentivirus develop a transient antibody response, but no disease develops.

#### Human T-cell Lymphotropic Viruses (HTLV-I) in Transverse Myelitis and Multiple Sclerosis

We have continued our studies of Jamaican neuropathy in Jamaica, and of Pacific (tropical) spastic paraparesis in the Tumaco area of southwestern Colombia on the Pacific coast. The Tumaco focus has a very uniform disease, which Dr. Gajdusek has, on three occasions, studied in the field in Colombia. In the earlier studies we found that patients had a much higher percentage of treponema positive spinal fluid than control patients, but in our most recent study there has been a striking decline in the rate of positivity, probably indicating that the earlier, not the newer, patients had yaws. On the other hand, we have now demonstrated an IgG antibody response in spinal fluid and serum to the human T-cell lymphotropic virus type I. This HTLV-I antibody-positive spinal fluid in the spastic paraparesis patients occurs in most of them, and in none of the controls with other neurological diseases. In the coastal area the seropositive titer in normal adults is very low, less than one-tenth found in the patients. In Jamaica, where the disease occurs

island-wide, the same finding has been reported by our group and we have confirmed the ELISA studies by Western blot and radioimmunoassay (RIA). We are also doing viral isolation studies on CSF from several Jamaican patients at this time; having already had an HTLV-I isolation from blood. In the study of spastic paraparesis in the Seychelles we have found similar seropositive rates for IgG antibodies to HTLV-I and the positivity rate for treponemal infection is no higher than in the controls.

New Japanese data indicates that a progressive chronic myelopathy is associated there with HTLV-I infection; thus, the findings in Martinique and our discoveries in Jamaica and the Colombian focus have been supported by those from Japan, the Seychelles and Trinidad, and serve to emphasize the need for intensive study of neurological involvement with HTLV-I infections, particularly in view of the fact that antibodies to a virus of this group have been found in cases of multiple sclerosis (MS). The similarity to MS of some of the histopathological changes in Jamaican patients has also provoked the thought that this group of viruses could play a role in producing varied manifestations of one disease process in different ethnic groups and regions--MS in temperate climates and tropical paraparesis in the tropical and subtropical areas.

It must be emphasized that the human T-cell lymphotropic viruses types I and II are not related virologically to the LAV-HTLV-III virus which is a lentivirus. They are not lentiviruses; they belong to a totally different sub-group of the retroviruses, and it is these viruses that may be producing chronic myelopathies; therefore, the problem needs much further clarification and investigation.

We are now studying intensively MS tissues including CSF and sera for HTLV-I antibody and frozen MS brain tissues for the presence of virus subunits, using in situ DNA hybridization to search for copies of the virus genome and fluorescent antibody techniques to search for the presence of subunit proteins of the virus.

#### Viliusk Encephalomyelitis in Yakut People in Siberia, USSR

We have just published a definitive bibliography of references on this disease since most reprints are unfamiliar to English-speaking neurologists. A complete review of this disease in English has been submitted to Brain. A detailed and comprehensive report of Viliusk encephalomyelitis (VE) is being prepared reporting the unique CNS pathology.

Analysis of clinical descriptions of 248 VE cases and a comprehensive clinical characterization of the disease has been made in comparison with the results of neuropathological study of 64 cases. VE geographical distribution and epidemiological features of the disease based on highly verified clinical material were also studied. A hypothesis of an infectious disease with a strong inflammatory component, probably slow virus infection, is hypothesized. Further studies on neuropathological and etiological aspects of VE have been initiated.

#### Hantaviruses and Hemorrhagic Fever with Renal Syndrome

Our work on the viruses of hemorrhagic fever with renal syndrome (HFRS) continues on an international level. We were the first laboratory to demonstrate the presence of an HFRS-related virus (Prospect Hill) in native American rodents, and to demonstrate that the massive Chinese epidemics of the past two decades have been caused by viruses closely related to those isolated from HFRS patients in Korea and Siberia. Our involvement has also been

intensive with the study of HFRS in the Balkans, particularly Yugoslavia and Hungary, and in Scandinavia, where in a milder form, called nephropathica epidemica, hundreds of cases may occur in a given year. Last year China alone had 83,000 cases of HFRS, with between five and ten percent mortality in most centers--in some small groups of patients considerably higher mortality. Over ten percent of the patients develop an encephalopathy during the course of the disease, but more interestingly, the disease affects particularly the pituitary gland and the pituitary stalk where hemorrhages are usually found in autopsied material.

During the past year we may have identified the first case of clinical disease in the United States from the American rats which are affected with the Asian type of Hantaan virus of the HFRS group of Bunyaviridae. This was a fatal case in Texas, in which the severe form of the disease appeared in an adult male who had been exposed, in the classical pattern, to rats while sleeping outdoors for several weeks.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 01282-22 CNSS
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
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Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief LCNSS
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LAB/BRANCH Laboratory of Central Nervous System Studies, Intramural Research Program		
SECTION		
INSTITUTE AND LOCATION NINCDS, Bethesda, Maryland 20205		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12	8	4
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Studies of human biology of vanishing primitive societies focus on neurological development and learning patterns in diverse cultural experiments in the human condition found in such isolated groups. Opportunistic investigation of problems phrased by man in isolation is the basis of approach from which all our studies have evolved. Techniques of molecular biology, immunology, virology, endocrinology and biochemistry and field epidemiological, clinical, linguistic and behavioral studies in cultural isolates and genetic and/or geographically isolated primitive bands yield more easily interpretable data than in cosmopolitan societies. Data and specimens collected on expeditions to Micronesia, Polynesia, Solomon Islands, New Hebrides, New Guinea, Indonesia, South America, Asia and Africa are used. Studies on nutrition, reproduction, fertility, neuroendocrine influences on age of sexual maturation and aging, genetic polymorphisms, genetic distance, unusual and odd employment of the higher cerebral functions in language learning, cognitive styles, computation (calculation without words or numbers) and culturally modified sexual behavior elucidate alternative forms of neurologic functioning for man which we would be unable to investigate once the natural cultural experiments in primitive human isolates are amalgamated into the cosmopolitan community of man. Foci of high incidence of kuru, ALS/PP, epilepsy, spastic paraparesis, familial parkinsonism, other CNS degenerations, hysterical disorders, schizophrenia, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, asthma, chronic lung disease, malaria, filariasis, leprosy, cysticercosis, and other infections are investigated. Zoonoses such as hemorrhagic fever with renal syndrome in China, Japan, Korea, USSR, Scandinavia, and the Balkans are studied including these newly recognized Bunyawera viruses in the U.S. Acquired immune deficiency syndrome studied by our group in 1950-1960 have been reinitiated. Human evolution and adaptability to high altitude, excessively wet or arid climes, variable food supply, mineral deficiencies, toxic exposures and responses to severe diseases or social/psychological stress are under investigation in appropriate population isolates.		

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Dr. L.G. Goldfarb, Institute of Poliomyelitis and Viral Encephalitis, Moscow;  
Prof. Vera I. Il'yenko, All-Union Research Institute of Influenza, Leningrad;  
Prof. D.K. Lvov, D.I. Ivanovskii Institute of Virology, Moscow;  
Dr. Prokopii Andrevich Petrov, Iakut Ministry of Public Health, Iakutsk;  
Dr. Anatoli Alexandrovich Smordintsev, Leningrad; Dr. Victor Zhadanov,  
Ivanovskii Institute of Virology, Moscow.

UNITED STATES: Alabama--Dr. James Dutt, University of South Alabama, Mobile;  
Dr. Charles Hoff, University of South Alabama, Mobile; Dr. Wladimir Wertelecki,  
University of South Alabama, Mobile; Arizona--Dr. Tim Kuberski, National  
Institute of Arthritis, Metabolism, and Digestive Diseases, Phoenix;  
California--Mr. James Boykin, Valencia; Dr. L.L. Cavalli-Sforza, Stanford  
University, Palo Alto; Dr. David Lang, City of Hope Hospital, Duarte; Dr.  
Michael N. Oxman, V.A. Hospital, San Diego; Dr. Guy Pawson, University of  
California, San Francisco; Delaware--Dr. Roger Rodrigue, Wilmington;  
Georgia--Dr. Carol Ballew, University of Georgia, Athens; Hawaii--Dr. Arwin  
Diwan, University of Hawaii, Honolulu; Dr. Leon Rosen, Pacific Research Center,  
Honolulu; Don Rubinstein, University of Hawaii, Honolulu; Illinois--Judith  
Farquhar, University of Chicago, Chicago; Maryland--Dr. Paul Hoffman, University  
of Maryland, Baltimore; Dr. Richard T. Johnson, Johns Hopkins Hospital,  
Baltimore; Dr. Guy McKhann, Johns Hopkins University, Baltimore; Dr. Chris  
Plato, Gerontology Research Center, Baltimore; Dr. Constantine Sakles,  
University Hospital, Baltimore; Dr. Charles Wissemann, University of Maryland,  
Baltimore; Dr. K.V. Shah, Johns Hopkins University, Baltimore; Massachusetts--  
Dr. John Enders, Brookline; Mr. Peter Fetchko, Peabody Museum, Salem;  
Michigan--Prof. J.V. Neel, University of Michigan, Ann Arbor; Dr. Ernst A.  
Rodin, Lafayette Clinic, Detroit; Minnesota--Dr. Leonard Kurland, Mayo Clinic,  
Rochester; Dr. G. Albin Matson, Minneapolis;

Nevada--Dr. Warren V. Huber, V.A. Medical Center, Reno; New Jersey--Dr. Karl Maramorosch, Rutgers University, New Brunswick; Dr. Richard Masland, Englewood; New York--Dr. Robert Glasse, Queen's College, Flushing; Dr. Shirley Lindenbaum, The New School, New York; Dr. Ralph D. Peterson, New York Hospital-Cornell Medical Center, New York; Dr. Roger D. Traub, IBM Thomas W. Watson, Yorktown; Ohio--Dr. Richard Feinberg, Kent State University, Kent; Dr. Frank P. Saul, Medical College, Toledo; Dr. Arthur G. Steinberg, Case Western Reserve University; Pennsylvania--Dr. Paul T. Baker, Pennsylvania State University, University Park; Drs. Werner and Gertrude Henle, Children's Hospital of Philadelphia, Philadelphia; Rhode Island--Dr. Terrence E. Hays, Rhode Island College, Providence; Dr. John Strom, Rhode Island Hospital, Providence; Texas--Dr. Heather D. Mayor, Baylor University Medical School, Houston; Dr. Steven Wiesenfeld, Southwest Allergy Service, Inc., Midland; Washington--Dr. Ronald DiGiacomo, University of Washington, Seattle; Wisconsin--Dr. G.R. Hartsough, Great Lakes Mink Association, Pittsville; Dr. Richard F. Marsh, University of Wisconsin, Madison; Dr. Gabriel Zu Rhein, University of Wisconsin, Madison.

YUGOSLAVIA: Dr. A. Terzin, Department of Microbiology, Faculty of Medicine, Novisad; Prof. J. Vesenjck-Hirjan, Sveucilistau Zagrebu, Zagreb.

- Sub-Project I: Study of the development patterning of the human nervous system (cybernetics of human development).
- Sub-Project II: Human evolutionary studies in isolated primitive groups.
- Sub-Project III: Studies of isolated Micronesian populations.
- Sub-Project IV: Studies of isolated New Guinea populations.
- Sub-Project V: Studies of Australian Aborigines.
- Sub-Project VI: Studies of isolated New Hebrides and Solomon Islands populations.
- Sub-Project VII: Studies of Central and South American Indians.
- Sub-Project VIII: Developmental, genetic and disease patterns in primitive and isolated populations of Asia, Africa, Indonesia, Melanesia, Micronesia, Polynesia, South and Central America, and the Arctic.
- Sub-Project IX: Experimental developmental neuropsychiatrics in infantile programming: a empirical approach to the language of information input into the nervous system.
- Sub-Project X: Ciphers and notation for the coding of sensory motor data for neurological information processing.



- Sub-Project XI: Racial distribution and neuroanatomic variations in the structure of the human brain.
- Sub-Project XII: Studies of high incidence of neurological disease in specific racial and ethnic groups and in primitive, or geographically genetically, culturally, or socially isolated group population studies.
- Sub-Project XIII: Studies of high incidence of non-neurological disease in specific racial and ethnic groups and in primitive, or geographically genetically, culturally, or socially isolated group population studies.
- Project Description: Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures (are attached)

Publications: Listed on pages 25 - LCNSS/IRP through 28- LCNSS/IRP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 00969-22 CNSS																												
PERIOD COVERED October 1, 1985 through September 30, 1986																														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infection																														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 40%;">D.C. Gajdusek, M.D.</td> <td style="width: 30%;">Chief</td> <td style="width: 10%;">LCNSS</td> </tr> <tr> <td colspan="4">Others:</td> </tr> <tr> <td></td> <td>Clarence J. Gibbs, Jr., Ph.D.</td> <td>Deputy Chief</td> <td>LCNSS</td> </tr> <tr> <td></td> <td>David M. Asher, M.D.</td> <td>Research Medical Officer</td> <td>LCNSS</td> </tr> <tr> <td></td> <td>Paul W. Brown, M.D.</td> <td>Medical Director</td> <td>LCNSS</td> </tr> <tr> <td></td> <td>Ralph M. Garruto, Ph.D.</td> <td>Senior Research Biologist</td> <td>LCNSS</td> </tr> <tr> <td></td> <td>Richard Yanagihara, M.D.</td> <td>Medical Officer</td> <td>LCNSS</td> </tr> </table>			PI:	D.C. Gajdusek, M.D.	Chief	LCNSS	Others:					Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief	LCNSS		David M. Asher, M.D.	Research Medical Officer	LCNSS		Paul W. Brown, M.D.	Medical Director	LCNSS		Ralph M. Garruto, Ph.D.	Senior Research Biologist	LCNSS		Richard Yanagihara, M.D.	Medical Officer	LCNSS
PI:	D.C. Gajdusek, M.D.	Chief	LCNSS																											
Others:																														
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LAB/BRANCH Laboratory of Central Nervous System Studies, IRP, NINCDS																														
SECTION  																														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20205																														
TOTAL MAN-YEARS 24	PROFESSIONAL: 14	OTHER: 10																												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews																														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>Studies elucidate cause and pathogenesis of chronic degenerative CNS disorders with emphasis on MS, ALS, Parkinsonism-dementia, Parkinson's, Pick's, and Alzheimer's disease, Huntington's chorea, supranuclear palsy, other presenile dementias, spinocerebellar ataxias, epilepsy, chronic encephalitis with focal epilepsy, muscular dystrophies, chronic schizophrenia, autism, SSPE, PML, dialysis encephalopathy, and intracranial neoplasm. Even familial, apparently hereditary diseases may be slow virus infections. Subacute spongiform virus encephalopathies: kuru and Creutzfeldt-Jakob disease (CJD) of man; scrapie and mink encephalopathy are caused by unconventional viruses with unique properties posing important theoretical problems to microbiology and molecular biology; a major goal is elucidation of their structure and mechanisms of replication. Transmissible virus dementias are increasingly recognized worldwide causes of death: high incidence foci, transmission by corneal transplant or brain surgery, and occupational hazards from exposure to diseased or infectious brain. In order to determine the usual mode of infection with the virus, a worldwide epidemiological study of transmissible virus dementia (CJD) cases is underway with special attention to familial clusters of cases and with a quest for possible relationship of scrapie of sheep to the human disease.</p> <p>Familial and nonfamilial dementia and the dementias of senility are studied. The autoimmune responses to specific brain antigens in CNS diseases are under intensive investigation. DNA <i>in situ</i> hybridization and electrophoretic focusing partition of proteins along with enzymatic and hybridoma immunofluorescence and many other techniques are used to try to identify viral subunits and partial genomes in tissues in chronic diseases.</p>																														

## PRINCIPAL INVESTIGATORS: (continued)

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New Jersey--Dr. L. Epstein, New Jersey College of Medicine and Dentistry, Newark. New York--Dr. Samuel J. Ayl, The National Foundation March of Dimes, White Plains; Dr. Jordi Casals, Mt. Sinai School of Medicine, New York; Dr. Teresita S. Elizan, Mt. Sinai School of Medicine, New York; Dr. Scott Halstead, Rockefeller Foundation, New York; Dr. Asao Hirano, Montefiore Hospital, Bronx; Dr. John Hotchin, Department of Health, Albany; Dr. Imaharu Nakano, Montefiore Hospital and Medical Center, New York; Dr. Michael L. Shelanski, New York University Medical Center, New York; Dr. Roger D. Traub, IBM Thomas B. Watson Research Center, Yorktown Heights; Dr. James D. Watson, Cold Spring Harbor Laboratory, Cold Spring. Ohio--Dr. S.M. Chou, Cleveland Foundation, Cleveland; Dr. Maurice Victor, Metropolitan General Hospital, Cleveland. Texas--Dr. Samuel Baron, University of Texas, Galveston; Dr. Steven Wiesenfeld, Southwest Allergy Service, Midland. Virginia--Dr. J. L. Hourrigan, Arlington. Washington--Dr. Ellsworth C. Alvord, Jr., University of Washington, Seattle. Washington, D.C.--Dr. Harold Booker, Veterans Administration Central Office, Washington; Dr. John Kurtzke, V.A. Hospital, Washington; Dr. Frederick C. Robbins, National Academy of Science, Washington;

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YUGOSLAVIA: Dr. A. Gligic, Institute of Immunology and Virology, Beograd; Dr. Miha Likar, Mikrobioloski Institut, Ljubljana; Dr. D. Terzin, Institute of Virology, Serajevo; Prof. J. Vesenjsek-Hirjan, University of Zagreb, Zagreb.

- Sub-Project I: Attempts to isolate, identify and characterize transmissible agents from humans and animals with subacute degenerative diseases of the central nervous system: transmissible hereditary diseases, presenile and senile dementias of the sporadic and familial types and primary sclerosing and demyelinating diseases.
- Sub-Project II: Characterization and pathogenesis of kuru virus.
- Sub-Project III: Characterization and pathogenesis of Creutzfeldt-Jakob disease (transmissible dementia virus).
- Sub-Project IV: Scrapie: studies on the purification, physical and biological characterization and nature of the virus.
- Sub-Project V: In vitro cultivation of the viruses of the subacute spongiform virus encephalopathies in cell cultures.
- Sub-Project VI: Host range of susceptible laboratory animals to the viruses of the subacute spongiform virus encephalopathies.
- Sub-Project VII: Strain variations among the viruses of the subacute spongiform virus encephalopathies.
- Sub-Project VIII: Cell-fusing properties of the viruses of the subacute spongiform virus encephalopathies.
- Sub-Project IX: Resistance to radiation of the viruses of the subacute spongiform virus encephalopathies.
- Sub-Project X: Resistance to disinfectants of the viruses of the subacute spongiform virus encephalopathies.
- Sub-Project XI: Tissue and cell culture techniques used to unmask slow infection of man and animals using brain and viscera biopsy and early autopsy, bone marrow and peripheral leucocyte specimens.
- Sub-Project XII: The syncytium-forming viruses (simian and human foamy viruses).

- Sub-Project XIII: Studies on transformed human brain tissue in vitro and characterization of associated virus.
- Sub-Project XIV: Electron microscopic membrane studies of subacute spongiform virus encephalopathies.
- Sub-Project XV: Characterization and identification of new herpes viruses from explant cultures of tissues from subhuman primates.
- Sub-Project XVI: Studies on persistent asymptomatic cytomegalovirus infections of healthy rhesus monkeys.
- Sub-Project XVII: Focal movement disorders in rhesus monkeys following experimental infection with a strain of tick-borne encephalitis virus.
- Sub-Project XVIII: Fluorescent antibody studies on the intracellular localization and identification of virus antigens in vivo and in vitro in tissues from patients with subacute diseases of the central nervous system.
- Sub-Project XIX: Isolation and characterization of adenovirus from the urine of chimpanzees.
- Sub-Project XX: Development of serological and immunological test system for use in the study of slow infections of the central nervous system.
- Sub-Project XXI: Immune responsiveness of multiple sclerosis patients to established viral antigens by detection of specific antibodies in serum and cerebrospinal fluids collected serially during remission and exacerbation.
- Sub-Project XXII: Animal management and intercurrent diseases in subhuman primates on long-term studies of slow infections.
- Sub-Project XXIII: Studies to determine the possible presence of cryptic viral genomes in human brain tissues.
- Sub-Project XXIV: Sequential development of kuru-induced neuropathological lesions in spider monkeys.
- Sub-Project XXV: Studies on the isolation, characterization, identification and pathogenicity of type C viruses from human and animal tissues.
- Sub-Project XXVI: Biochemical studies of the etiology of amyotrophic lateral sclerosis and parkinsonism-dementia.
- Sub-Project XXVII: Study of mitochondrial mutants from scrapie-infected mouse brain cells.



- Sub-Project XXVII: Study of mitochondrial mutants from scrapie-infected mouse brain cells.
- Sub-Project XXVIII: Isolation and characterization of the etiological agent of Scandinavian nephro-nephritis epidemica.
- Sub-Project XXIX: The pathogenesis of Korean hemorrhagic fever virus and the elucidation of its biological and physical properties.
- Sub-Project XXX: Worldwide seroepidemiological evidence of antibodies in human populations to the virus of Korean hemorrhagic fever.
- Sub-Project XXXI: Development of an enzyme-linked immunoadsorbent (ELISA) test for the diagnosis and epidemiology of cystercercosis-induced epilepsy.
- Sub-Project XXXII: Studies on the cytochemical and morphological properties of neurons cultured in vitro.
- Sub-Project XXXIII: Development of immunological markers for the detection of autoantibodies to neurofilaments in the sera of patients with subacute spongiform encephalopathies.
- Sub-Project XXXIV: Studies to determine the neurophysiological changes of neurons in vitro infected with CJD.
- Sub-Project XXXV: Effects of the subacute spongiform viruses on nerve cells grown in vitro.
- Sub-Project XXXVI: In vivo and in vitro studies to determine the etiology of myasthenia gravis, Viliuisk encephalomyelitis and ALS-PD in high incidence foci of the Western Pacific.
- Sub-Project XXXVII: Neurophysiological study of animals experimentally infected with subacute spongiform virus encephalopathies.
- Sub-Project XXXVIII: Studies on in vivo pathogenicity of the retroviruses related to AIDS: HTLV (Gallo); French LAV-LOISEAU virus (Montagnier)
- Sub-Project XXXIX: Attempts to transmit or isolate in vitro an etiological agent from AIDS, from pre-AIDS patients with lymphadenopathy syndrome, and from encephalitis associated with AIDS.
- Sub-Project XXXX: Isolation and characterization of "unconventional viruses" (CJD) from multiple lots of human pituitary growth hormone.
- Sub-Project XXXXI: Epidemiology of progressive degenerative disease of the CNS in recipients of human pituitary growth hormone.

Sub-Project XXXXII: Development of procedures to exclude "unconventional viruses" from preparations of human pituitary growth hormone.

Sub-Project XXXXIII: Preparation and characterization of synthetic polypeptides for scrapie, kuru, CJD and core protein of amyloid plaques in Alzheimer's disease.

Sub-Project XXXXIV: Studies on the effects of altered slow axonal flow in the pathogenesis of subacute progressive degenerative disease of the nervous system.

Sub-Project XXXXV: Studies on the deposition and distribution of heavy metals and essential minerals in central nervous system tissue from patients with progressive neurodegenerative disorders.

Project Description: Chronic Central Nervous System Disease Studies (described fully on pages 1-LCNSS/IRP through 6-LCNSS/IRP).

The projects (I through XXXXV) listed herein, as itemized in the Project Reports of previous years, have continued throughout this year and have been expanded, as are reflected in the extensive list of publications. Contractural phases of this work are being conducted at University of Southwestern Louisiana, New Iberia Research Center, New Iberia, Louisiana.

Publications: Pages 25-LCNSS/IRP through 28-LCNSS/IRP

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CONTRACTS

University of Southwestern Louisiana  
New Iberia Research Center  
New Iberia, Louisiana

Contract #N01-NS-8-00931

\$91,660.00

Program Resources, Inc.  
(Administration by NCI)

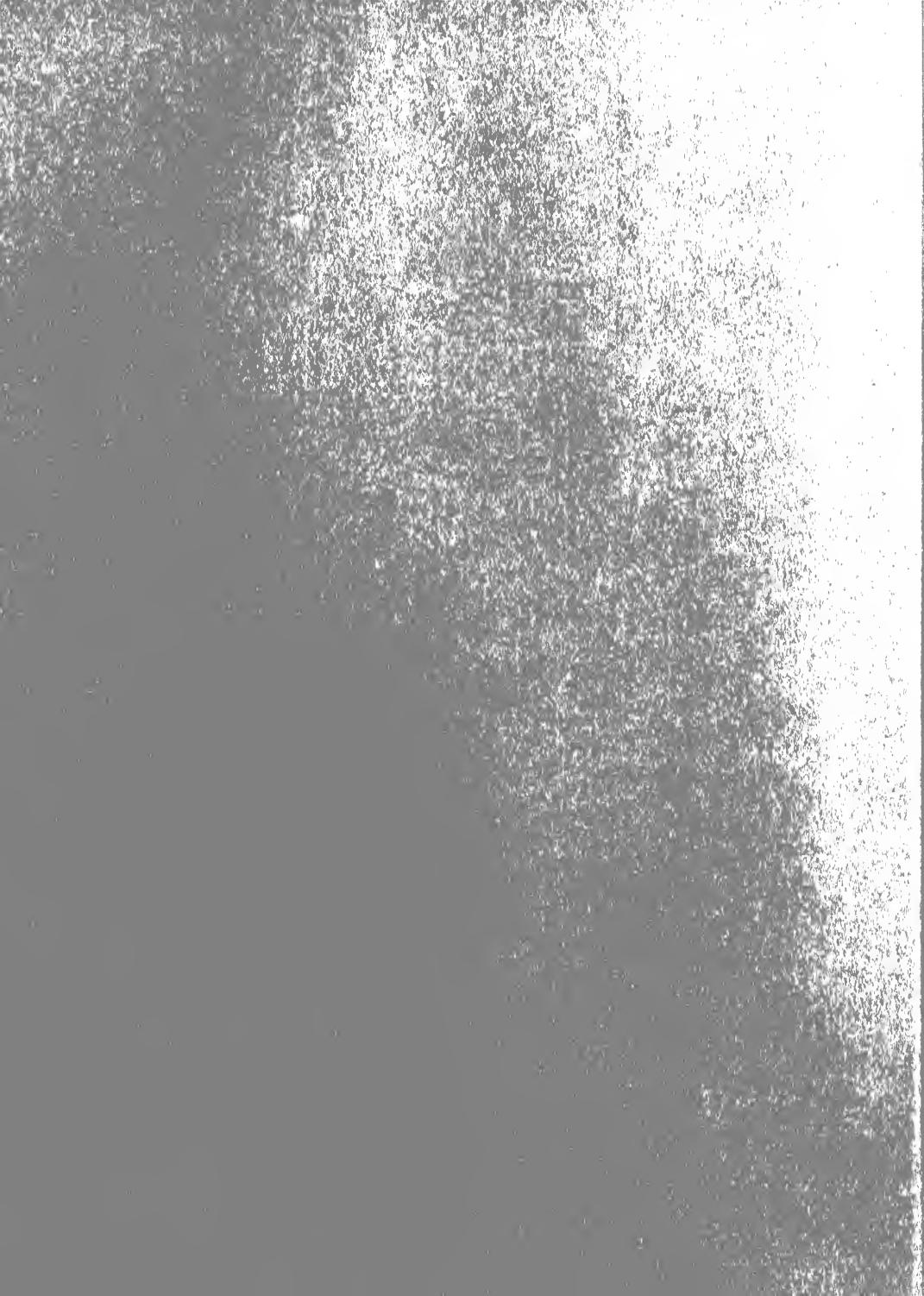
Contract #N01-CO-75380

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ANNUAL REPORT

October 1, 1985 through September 30, 1986

Laboratory of Experimental Neuropathology

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## ANNUAL REPORT

October 1, 1985 through September 30, 1986

Laboratory of Experimental Neuropathology, IRP  
National Institute of Neurological and Communicative  
Disorders and Stroke

Henry deF. Webster, M.D., Chief

The Laboratory of Experimental Neuropathology (LENP) includes the Cellular Neuropathology Section (CN) and the Neurotoxicology Section (NT). The main goal of the Laboratory's research program is to investigate cellular mechanisms of myelin breakdown, especially those that are directly related to multiple sclerosis and other human demyelinating diseases. Other closely coordinated efforts use biochemical, biophysical and neuropharmacological methods to explore gangliosides, membrane fusion mechanisms and neurotoxic actions. Major discoveries during the past year include: (1) a mouse model for producing latent herpes simplex virus type 2 (HSV-2) infection which can be reactivated to produce infection in the CNS, PNS and genital tract, (2) the demonstration of cross-reactivity of the C-terminal sequence of JC virus T-antigen with hamster antibody to myelin basic protein, and (3) light and electron microscopic detection of P<sub>0</sub> messenger RNA in the cytoplasm of Schwann cells and along margins of developing myelin sheaths after *in situ* hybridization with a P<sub>0</sub> biotinylated cDNA probe. The following summary describes these discoveries and the most significant findings obtained in FY 1986 LENP projects.

### Cellular Neuropathology Section (CN)

#### 1. Herpes Simplex Virus Type 2 (HSV-2) Pathogenesis and CNS Demyelination

This project has three aims: (i) To define disease produced by HSV-2 in experimental mouse models, including primary and reactivated infections, (ii) To relate neurological disease, particularly CNS demyelination, to factors that influence the outcome of disease, including host age, virus dose, inoculation route, immunological factors, and host and virus genetics, and (iii) To use insights gained from these models to refine and test a working hypothesis that HSV-2 may have an etiological role in multiple sclerosis (MS).

Published background studies from this laboratory include: (i) The first evidence that HSV-2 can produce multifocal CNS demyelination in mice which mimics certain features of MS pathology. One HSV-2 strain we used was originally isolated from the midbrain of an MS patient. (ii) Evidence that HSV-2 can produce non-fatal CNS demyelination by a natural genital route of infection. (iii) Evidence that thymic cortical necrosis and virus presence in lymphoid tissues segregate with severe CNS disease in acute infections, suggesting that immunosuppression may be induced as a result of HSV-2 infection. (iv) Evidence suggesting that tract-associated demyelinative lesions arise from a minimal neuronal infection with axonal transport, axon-to-glial spread, and amplification of infection in white matter. (v) A comparison of the epidemiologies of MS and of HSV infections, which suggests that MS could be a low-frequency complication of HSV-2 infection in persons lacking previous protective HSV-1 immunity. These studies are important because they address major plausibility issues which any specific etiological hypothesis for MS must satisfy.

Experiments undertaken, in progress, or completed in FY 1986 address important unresolved questions, including (i) whether, where, and under what conditions HSV-2 can establish latency or reactivate in the PNS or CNS, (ii) whether recurrent CNS demyelination is a consequence of reactivation, and (iii) whether infection is capable of inducing immunosuppression, either by lymphoid cell infection or by other means.

These experiments show that genital infection can establish a more extensive latent infection of dorsal root ganglia (DRG) than previously recognized. Latent infection is established in lower thoracic and lumbosacral DRG. Further, reactivated infection can be efficiently and reproducibly induced using immunosuppressive treatment. With reactivation, 2 discrete groups of DRG contain viral antigen: T-9 to L-2, and L-6 to S-2, apparently corresponding to hypogastric and pudendal innervation of the genito-urinary tract and external genitalia. Viral antigen can also be found in satellite cells and endoneurial cells in DRG, distal roots, and occasionally in cells of the spinal cord. While many mice develop foci of CNS white matter demyelination during primary infection, recurrent demyelinative lesions have thus far not been seen in this reactivation model. This is the first study to analyze the nervous system effects of HSV-2 reactivation.

In another study, viral effects on lymphoid tissues from animals at different ages are examined by comparing virus isolation, viral antigen, and ultrastructural observations to those obtained using biotinylated cDNA probes and *in situ* hybridization methods. In permissive cells from young animals, infection of a broad spectrum of lymphoid cells can be demonstrated by traditional methods, as well as with *in situ* hybridization. These studies should provide a useful means to look for nonproductive or latent infection of lymphoid and neural tissues.

Other studies in progress include: (i) Examination of CNS remyelination in a mouse model of genital infection and demyelination, (ii) A study of viral antibodies in optic neuritis, and (iii) A search for viral antigen in MS tissues.

## 2. P<sub>0</sub> Glycoprotein Messenger RNA and Expression of P<sub>0</sub> in Developing Peripheral Nervous Tissue.

It is now widely recognized that *in situ* nucleic acid hybridization techniques offer important new approaches for studies of how synthesis of cellular constituents is regulated in normal tissue and what changes are associated with lesions found in human and experimental diseases. Few observations on distributions of messenger RNAs for myelin proteins have been reported and in these, the relative amounts of radiolabelled probes found in relatively large areas on CNS light microscopic sections reflect previously known patterns of myelin protein expression and myelin sheath formation in developing CNS tracts (Kristensson et al., 1986; Trapp et al., 1986).

Since the spatial resolution of the light microscopic autoradiographic methods used in reported studies could not define intracellular distributions of probe in single Schwann cells, our first goal in this new project was to develop a technique suitable for intracellular light and electron microscopic localization of P<sub>0</sub> mRNA within developing, mature and diseased Schwann cells. A biotinylated P<sub>0</sub> cDNA was used to probe sections that subsequently were examined by both light and electron microscopy. P<sub>0</sub> messenger RNA

was detected light microscopically in perikarya of myelin-forming Schwann cells and this localization has recently been confirmed in preliminary electron microscopic experiments.  $P_0$  mRNA also has been found on developing myelin sheaths and often was concentrated along sheaths' inner and outer margins. Our results show that the distribution of  $P_0$  messenger RNA can now be studied at the cellular instead of the tissue level by using a biotinylated cDNA probe instead of the radiolabelled probes used previously. This represents a substantial improvement in methodology, and observations made with this technique are currently being correlated with light and electron microscopic immunocytochemical results that describe the appearance and distribution of  $P_0$  glycoprotein immunoreactivity during development.

### Neurotoxicology Section (NT)

The Neurotoxicology Section seeks to elucidate basic molecular mechanisms of immunologic, virologic, and neurotoxic damage to the CNS, with a focus on membrane interactions in general, and on the myelin membrane in particular. The main approaches currently are (a) MBP structure and function studies, with delineation of possible relationships between MBP and viral proteins such as the T-antigen of papovaviruses JC and BK; (b) studies of the multiple roles of gangliosides, which are traditional target sites for toxins in the CNS, especially as modulators of protein kinase activity in myelin; and (c) stopped flow studies of membrane fusion events which can probe the role of MBP in aggregating and fusing vesicles, and thus of phosphorylation and various toxins in regulating the activity of a membrane fusogen such as MBP.

#### 1. Myelin basic protein, JC virus T-antigen, and human demyelinating disease.

Theories of multiple sclerosis (MS) etiology are primarily three: (a) a direct cytopathic effect of a latent or persistent virus in the CNS, (b) induction of an autoimmune response to a normal myelin constituent, such as MBP, by an infectious event, probably a viral infection, and (c) a combination of (a) and (b) in which the target of immunologic attack in the CNS is the viral antigen itself, rather than a normal myelin constituent. Our interest in T-antigen as an inducer and/or target of anti-myelin activity originated from the observation of a shared amino acid sequence which includes the central triproline region of MBP and the extreme C-terminus of JC (and BK) virus T-antigens. This sequence relationship was detailed in last year's report. Interestingly, JC virus, which causes the rare demyelinating disease, progressive multifocal leukoencephalopathy (PML) in which oligodendrocytes are productively infected, could operate by any of the three mechanisms outlined above. The closely related BK virus, on the other hand, which shares the same C-terminal sequence with MBP, could operate only by mechanism (b), because it is known to latently infect the kidney, as does JCV, but has never been found to infect the brain. During the past year progress in elucidating potential mechanisms of myelin damage by JCV or BKV has included the following: First, an antibody to MBP raised in a hamster was shown to be blocked by a synthetic decapeptide corresponding to the JCV T-antigen C-terminus. This evidence for the existence of an immunological cross-reaction between MBP and the T-antigen sequence supports an autoimmune or "molecular mimicry" mechanism. Second, a possible direct cytopathic effect for oligodendroglial cells was proposed to result from the shared protein kinase recognition site, in that the Thr residue preceding both triprolyl sequences is known to be phosphorylated. Appearance in the cell of T-antigen as an alternate substrate for this and possibly other protein kinases could seriously interfere with normal phosphorylation and function of MBP. Evidence was obtained

using a synthetic peptide corresponding to the MBP triproline region that this sequence is, in fact, a recognition site for protein kinase C. Thus the two sequences might compete for the same enzyme. However, while T-antigen was readily detected in PML brain by the peroxidase-antiperoxidase immunocytochemical method using frozen tissue sections to avoid denaturation of the T-antigen, and evidence was obtained that many small oligodendrocytes produce T-antigen alone without virus production, we could not find evidence with these methods for a latent infection expressing T-antigen in MS brains. We are currently developing more sensitive immunocytochemical methods for T-antigen detection with which to re-examine this question.

## 2. Myelin Basic Protein Conformation Studies

Myelin basic protein has been considered to exist in solution as a random coil with no long range order. Our recent experiments show that the naturally fluorescent amino acids of MBP exist in ordered structure. The protein binds ANS derivatives with  $K_d$ 's of  $10^{-6}$  to  $10^{-5}$  M. Porphyrins also bind with  $K_d$ 's of  $\sim 10^{-8}$  competitively displace ANS. These results argue for a high degree of 3-dimensional structural specificity of MBP.

## 3. Gangliosides and Protein Phosphorylation: Roles in CNS Function, Growth, Differentiation, and Neurotoxicity.

This project is aimed at determining whether there is a direct relationship between the modulation of gangliosides and protein phosphorylation on neural activities. Gangliosides, a class of sialic acid-containing glycosphingolipids, are major constituents of the membrane components in the CNS and have been implicated to play an important role in certain neuronal functions such as neurotransmission, neurite outgrowth, synaptogenesis, neuronal regeneration, differentiation and development. The exact mode of action of these glycolipids, however, is unknown. Current research in this laboratory attempts to define a molecular basis through which gangliosides may mediate their effects. We found that gangliosides have profound modulatory effects on the phosphorylation of specific proteins in both synaptosomes and myelin. Because posttranslational modification of proteins and peptides through phosphorylation and dephosphorylation is of paramount importance in the regulation of numerous biological processes, it seems likely that gangliosides may act as one of the mediators for this mechanism. A novel ganglioside-dependent protein kinase has been identified, partially purified and characterized. Activation of this enzyme by gangliosides, especially polysialogangliosides, is specific and could not be mimicked by other second messengers including cyclic nucleotides,  $Ca^{2+}$  and phospholipid or calmodulin. The substrate specificity of the ganglioside-dependent protein kinase also is distinct from cAMP-dependent,  $Ca^{2+}$ /phospholipid-dependent, and  $Ca^{2+}$ /calmodulin-dependent protein kinases. Stimulation of the kinase activity by gangliosides is not time-dependent and  $Ca^{2+}$  is not absolutely required in this process, suggesting that proteolysis and  $Ca^{2+}$ /ganglioside complexes are not involved. The ganglioside-dependent protein kinase can not phosphorylate tyrosine residues but can autophosphorylate itself at a serine residue. The physiological significance of these results are under investigation. Gangliosides also can activate protein kinase C activity in vitro, provided phosphatidylserine is present. Thus besides phosphatidylinositol



turnover, perturbation of membrane structures which results in changes in the contents or microenvironment of gangliosides may provide yet another means of activating this pivotal  $\text{Ca}^{2+}$ /phospholipid-dependent kinase.

The state of phosphorylation of myelin basic protein in CNS myelin is highly regulated, albeit the functional significance of this modification and its relationship to demyelinating diseases have not been established. We found that exogenous addition of gangliosides to purified myelin could either inhibit the phosphorylation state. Staphylococcal A-toxin, a neurotoxin which has previously been shown to cause demyelination in vivo, also can stimulate the phosphorylation of myelin basic protein in purified myelin. In vitro experiments revealed that this toxin has a stimulatory effect on the activity of cAMP-dependent, but not  $\text{Ca}^{2+}$ /phospholipid-dependent, protein kinase. The possible roles of myelin basic protein phosphorylation in the formation and maintenance of structural integrity in myelin are currently being pursued.

#### 4. Neurotoxicity Mechanisms Studied in a Chromaffin Cell System

The chromaffin cell provides a well-characterized system for investigating molecular and cell-surface mediated mechanisms of neurotoxin action. Since several neurotoxins of interest to neurology are divalent cations (lead, manganese, copper, etc.) and since the storage vesicles of these cells, the chromaffin granules, contain high concentrations of calcium, these preparations have been investigated to determine the effect of toxic cations on calcium-mediated storage and release processes. Release of neurotransmitters and neuromodulators from their storage organelles takes place by exocytosis, a process in which the influx of calcium into the cell or nerve terminal triggers the fusion of the storage granule with the cell plasma membrane. The membrane fusion events can be modeled by studying the calcium-promoted fusion of artificial or biological membranes with each other.

A new multichannel, computer-controlled stopped-flow rapid mixing spectrometer has been constructed to study the kinetics of these reactions. Our previous stopped-flow mixing studies have shown that the kinetics of both aggregation and fusion of small vesicular structures (artificial lipid vesicles, neurotransmitter storage granules, etc) follow second order kinetics with fusion being aggregation rate limited. We have previously shown that this process can be followed in real time using a fluorescence resonance energy transfer (RET) assay. As a model for this process, we have been investigating fusion of artificial phospholipid bilayer membrane vesicles. Using the same assay, based on resonance energy transfer from NBD-PE to N-Rh-PE, we have previously demonstrated by stopped-flow mixing techniques that the fusion of these vesicular structures can be described as the sum of several bimolecular rate reactions. In the course of this investigation it became apparent that the NBD-PE probe is highly sensitive to the presence of divalent cations. This manifests itself as a change in the quantum yield of the probe which occurs faster than the aggregation of the probe-containing vesicles and follows pseudo-first order kinetics. This contaminating process must be removed from the data before analysis of fusion can be performed.

We have developed a new assay based on the ability of pyrene to form fluorescent excimers as a function of the separation distance between the probe molecules which not only overcomes this problem but produces excellent results which are easily checked against predicted behavior. We find that the spontaneous exchange rate for this probe between PC bilayers is very slow. Similar results were obtained for PS:PE 1:1 bilayers. This slow exchange, in contrast to rapid exchange of chain labelled NBD-PC probably is due to the tremendous increase in hydrophobicity of the pyrene versus the NBD molecule.

The ratio of monomer to excimer fluorescence (E/M ratio) from pyrene-PC incorporated into PS:PE bilayers is linear with increasing probe concentration up to 6 mol percent probe. Fusion of labelled with unlabelled PS:PE vesicles linearly reduces the E/M ratio as would be predicted by increased distance between fluorophores experienced by fusion. Thus stochastic predictions of extent of fusion can easily be checked experimentally.

Stopped-flow mixing experiments show initial  $Mg^{2+}$ -promoted fusion of PS:PE vesicles follow second-order, aggregation rate-limited kinetics, as was previously found for  $Ca^{2+}$  using the NBD-PE to rhodamine-PE resonance energy transfer assay. Furthermore, the change in E/M measured for total fusion as well as the amplitude for the monomer to dimer conversion (calculated by multiparameter fitting of the stopped flow data) agree with those predicted from the stochastic model. There is no prolonged or extensive lateral phase separation involved in the calcium- or magnesium-promoted fusion of PS:PE vesicles.

The new assay solves problems encountered in using the NBD-PE/rhodamine-PE assay. NBD-PE fluorescence is shown to be sensitive to changes in the headgroup environment caused by ion binding. It will greatly increase the precision of our membrane fusion assays. Besides being more tractable, it offers the advantage of having to label only one of two membranes in an "asymmetric" system, such as fusion of storage organelles with cell plasma membranes. It has already been applied with success to studies of myelin basic protein-catalyzed fusion of phospholipid bilayer membrane vesicles (see project No. ZO1 NS 02451-06).

#### 5. Anticonvulsant Drugs, Seizure Disorders, and Specific Adenosine Receptors

The mechanism of action of the anticonvulsants of the barbiturate and benzodiazepine class appears to be an interaction between those therapeutic agents and benzodiazepine receptors in the central nervous system. On the other hand, the mechanism of action of carbamazepine, currently one of the most frequently prescribed therapeutic anticonvulsant agents, is unknown. Since adenosine and adenosine analogs have anticonvulsant effects in rat and mouse, and adenosine antagonists display convulsant activity, we have been investigating the possibility that carbamazepine exerts its anticonvulsant effect by binding to central adenosine receptors.

The nanomolar concentrations of adenosine are inhibitory to adenylate cyclase at  $A_1$  adenosine receptors in the central nervous system and other tissues. Studies characterizing these  $A_1$  adenosine receptors, and drug interactions with them, can be implemented by measuring the binding of radiolabelled adenosine agonists and antagonists to these receptors. Adenosine's stimulatory actions on adenylate cyclase however, occur at micromolar concentrations at  $A_2$  adenosine receptors and are best measured by quantitating the accumulation of cAMP in preparations of tissue slices. We have studied the effects of carbamazepine on  $A_1$  adenosine receptors by using the adenosine analog, [ $^3H$ ]cyclohexyladenosine, in classical receptor-ligand interaction studies with brain membranes from Sprague Dawley rats. The effects of carbamazepine on  $A_2$  adenosine receptors have been studied by measuring the accumulation of radioactive cAMP in [ $^3H$ ]adenine-labeled cerebral cortical brain slices from male Hartley strain guinea pigs.

Carbamazepine appears to be more potent at the A<sub>1</sub> adenosine receptor than at the A<sub>2</sub> adenosine receptor. Interactions of carbamazepine with central nervous system A<sub>1</sub> adenosine receptors occur at therapeutic concentrations, but equivalent interactions at A<sub>2</sub> adenosine receptors require fourfold higher concentrations. Carbamazepine is clearly an antagonist at the A<sub>2</sub> adenosine receptor but its role at A<sub>1</sub> adenosine receptors is less well defined. Despite extensive studies comparing and contrasting carbamazepine's actions on A<sub>1</sub> adenosine receptors with the actions of known agonists, antagonists, and adenosine analogs we have not been able to categorize carbamazepine as either a pure agonist or a pure antagonist at A<sub>1</sub> adenosine receptors. The potency of various adenosine analogs, agonists, and antagonists to inhibit [<sup>3</sup>H]cyclohexyladenosine binding to rat brain A<sub>1</sub> adenosine receptors at the higher affinity nanomolar site versus the lower affinity nanomolar site can be described graphically by linear plots. However, the relationships for adenosine agonists and antagonists are clearly different. Notably, data on carbamazepine's inhibitory actions are more closely aligned with data from known antagonists. The relationship between carbamazepine and adenosine receptors requires further investigation. Such studies will promote a better understanding of the actions of this therapeutic anticonvulsant and clarify further directions for the development of future anticonvulsant medications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02549-05  
LENP

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Herpes Simplex Virus Type 2 Infection, CNS Demyelination and Multiple Sclerosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.R. Martin	Medical Officer	LENP	NINCDS
Others: S. Suzuki	Visiting Fellow	LENP	NINCDS
D. Soffer	Visiting Scientist	LENP	NINCDS
H. Webster	Chief	LENP	NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Section on Experimental Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.7

PROFESSIONAL:

2.3

OTHER:

2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project seeks to define the spectrum of acute and recurrent CNS and peripheral disease, including CNS demyelination, which is produced in experimental herpes simplex virus type 2 (HSV-2) infection, and to refine and test a hypothesis which relates HSV-2 infection to the human demyelinating disease, multiple sclerosis (MS). Our previous studies suggest that several major features of HSV epidemiology and pathology are consistent with the hypothesis that HSV-2 is etiologic in MS.

During FY 1986, studies published or submitted provide evidence which further defines the spectrum of experimental disease produced by HSV-2 in the CNS and in lymphoid tissues. These studies provide insights into human infections and disease which HSV-2 is known to cause, and suggest how HSV-2 could produce a CNS demyelinating disease. Specifically, they show that:

1) When mice with latent HSV-2 infections, previously established by a genital route, are immunosuppressed, virus can again be isolated from the genital tract. In this reactivation model, virus can be demonstrated by virus isolation or antigen detection methods in the spinal cord, brain and spleen tissues. Virus is also found in 2 distinct groups of dorsal root ganglia, where viral antigen is present in neurons, endoneurial cells, and axons. In this model, viral latency, reactivation, nervous system disease can be studied, and vaccine efficacy can be tested.

2) At early stages of primary infection, virus is first detected in sensory roots, then spreads to the spinal cord. This study provides further evidence that HSV-2 spreads to the CNS chiefly via peripheral nerve roots, probably in the intra-axonal compartment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01995-14  
LENP

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphological Studies of Myelin Formation, Breakdown and Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. deF. Webster	Chief	LENP	NINCDS
Others:	L. Lampertth	Visiting Fellow	LENP	NINCDS
	J.T. Favilla	Biologist	LENP	NINCDS
	L. Manuelidis	Assoc. Prof.	Yale University	
	G. Lemke	Scientist	Salk Institute	
	M. Graeber	Scientist	Max Planck Institute	

COOPERATING UNITS (if any)

Neuropathology Section, Department of Neurosurgery, Yale Medical School, New Haven, Conn; Salk Institute, San Diego, California; Max Planck Institute for Psychiatry, Munich, Fed. Rep. Germany

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Section on Cellular Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.6

PROFESSIONAL:

2.6

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long range goal of this project is to combine in situ nucleic acid hybridization techniques and immunocytochemical methods to study cellular mechanisms of myelin formation, breakdown and regeneration. Current studies and major findings are: (1) Localization of mRNA encoding for P<sub>0</sub> glycoprotein in sections of developing peripheral nervous tissue by in situ hybridization. Aldehyde-fixed light microscopic vibratome and semithin sections of trigeminal nerves and ganglia from 15 day old rats were treated with biotinylated P<sub>0</sub> cDNA according to procedures described by Manuelidis (1985) and Lemke and Axel (1985). Reaction product that localized P<sub>0</sub> mRNA was detected light microscopically in perikarya of myelin-forming Schwann cells. It also was found along the inner and outer margins of developing myelin sheaths. Our results show that use of a biotinylated P<sub>0</sub> probe provides more precise localization of P<sub>0</sub> mRNA than has been obtained with the use of paraffin sections and radio-labelled probes. Experiments in progress include electron microscopic studies of P<sub>0</sub> mRNA localization. (2) Intracellular Distribution of P<sub>0</sub> glycoprotein in developing Schwann cells determined by electron microscopic immunogold staining. A method described by Graeber and Kreutzberg (1985) has been modified to preserve the fine structure of myelin-forming Schwann cells in LR White-embedded developing trigeminal nerves. Thin sections have been immunostained with a polyclonal antiserum. In electron micrographs, anti-P<sub>0</sub> immunoreactivity is present on developing myelin lamellae. Further observations, including those produced with monoclonal anti-P<sub>0</sub> are in progress. (3) Quantitative morphometric study of PNS nerve fibers in the mouse mutant, shiverer. Measurements in mutants and controls have shown that myelinated nerve fibers in shiverer PNS are significantly smaller in size, more densely packed and possess thin myelin sheaths relative to axon caliber. These results suggest mild PNS immaturity, growth retardation and hypomyelination in a mutant thought previously to have essentially normal myelinated fibers in the PNS.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02550-05

LENP

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Immunologic mechanisms in virally-induced CNS demyelination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G.L. Stoner	Senior Staff Fellow	LENP	NINCDS
Others: H.deF. Webster	Chief	LENP	NINCDS
K.-F.J. Chan	Senior Staff Fellow	LENP	NINCDS
S.J. Morris	Guest Researcher	LENP	NINCDS
D. Soffer	Visiting Scientist	LENP	NINCDS
C. F. Ryschkewitsch	Medical Technologist	LENP	NINCDS

## COOPERATING UNITS (if any)

Dept. of Medical Microbiology, Univ. of Wisconsin Med. School, Madison (D.L. Walker) Dept. of Chemistry, Rhodes College, Memphis, TN (R.D. Gilliom)

## LAB/BRANCH

Laboratory of Experimental Neuropathology

## SECTION

Neurotoxicology Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

1.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previously the prediction of B-structure in myelin basic protein (MBP) led to the suggestion that MBP shares a threonine protein kinase recognition sequence with the C-terminus of the T-antigen, a regulatory, DNA-binding protein of the JC, BK, and SV40 viruses. This observation has raised important questions relating to (i) possible evolutionary relationships of MBP and the T-antigen sequences, (ii) the functional relationship of these sequences and the role of phosphorylation in these proteins, (iii) the possibility of immunological cross-reactions with potential for immunopathology in the CNS, and (iv) the pathological effects of latent JCV infection of the oligodendrocyte, the myelin-forming cell in the CNS. This past year a possible evolutionary relationship of the MBP site and the T-antigen C-terminus has been established. In addition, studies by K.-F. J. Chan (then of NICHD, ERR) established the Arg-Thr-Pro-Pro-Ser sequence of MBP as a protein kinase recognition site. Further, fluorescence studies in collaboration with S.J. Morris have provided strong experimental evidence for an organized structure in MBP which is consistent with the model originally predicted. Experiments utilizing antibodies raised in hamsters have established the existence of a cross-reactivity between the MBP and T-antigen tripolyol sequences. We have developed an ELISA inhibition assay to show that a synthetic decapeptide corresponding to the C-terminus of the JCV T-antigen strongly inhibits the binding of a hamster antiserum to MBP. A second antibody determinant in hamsters was identified in the peptide around Trp-117 which contains the encephalitogenic determinant for guinea pigs. Expression of T-antigen in human brain has so far been established for ordinary progressive multifocal leukoencephalopathy (PML) and for the severe form of PML identified in brains of some AIDS patients. The interaction between JCV and HTLV-III which leads to a high incidence and severe form of PML in AIDS remains to be identified. An initial search for T-antigen in multiple sclerosis (MS) brain using these methods was negative. However, more sensitive methods have now been developed, and are being applied to both frozen brain and kidney tissue from MS patients.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02451-06 LENP												
PERIOD COVERED October 1, 1985 through September 30, 1986														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cellular and Molecular Approaches to Neurotoxicology														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: S.J. Morris</td> <td style="width: 33%;">Guest Worker</td> <td style="width: 33%;">LENP NINCDS</td> </tr> <tr> <td>Others: D. Bradley</td> <td>Chemist</td> <td>LENP NINCDS</td> </tr> <tr> <td>G.L. Stoner</td> <td>Senior Staff Fellow</td> <td>LENP NINCDS</td> </tr> <tr> <td>A.T. Campagnoni</td> <td>Professor</td> <td>UCLA Medical School</td> </tr> </table>			PI: S.J. Morris	Guest Worker	LENP NINCDS	Others: D. Bradley	Chemist	LENP NINCDS	G.L. Stoner	Senior Staff Fellow	LENP NINCDS	A.T. Campagnoni	Professor	UCLA Medical School
PI: S.J. Morris	Guest Worker	LENP NINCDS												
Others: D. Bradley	Chemist	LENP NINCDS												
G.L. Stoner	Senior Staff Fellow	LENP NINCDS												
A.T. Campagnoni	Professor	UCLA Medical School												
COOPERATING UNITS (if any) Mental Retardation Research Center, UCLA Center for the Health Sciences, Los Angeles, CA 90024														
LAB/BRANCH Laboratory of Experimental Neuropathology														
SECTION Neurotoxicology Section														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.5	OTHER: 0.1												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>Neurotoxins are known to disrupt the structure of myelin. The membranes of this ubiquitous material of the nervous system contain three major proteins. <u>Myelin basic protein</u>, which accounts for 30 percent of CNS myelin proteins, has no known physiological function, although injection of purified MBP will cause <u>Experimental Allergic Encephalomyelitis</u>, considered by some as a model for <u>Multiple Sclerosis</u>. A molecular model for the structure of MBP generates a series of testable predictions. We have been examining the structural properties of MBP using fluorescence and optical spectroscopy. Contrary to many reports, we find evidence for extensive long range structural specificity of MBP in agreement with the model. We find that a subfraction of isolated MBP molecules bind <u>heme</u> and other <u>porphyrins</u> with affinities on the order of <math>10^{-8}</math> M. These molecules also bind <u>ANS</u> derivatives with affinities on the order of <math>10^{-7}</math> M. Heme rapidly displaces bound <u>bis</u>-ANS.</p> <p>These studies may lead to a rapid, more precise functional assay for MBP than the induction of EAE.</p>														

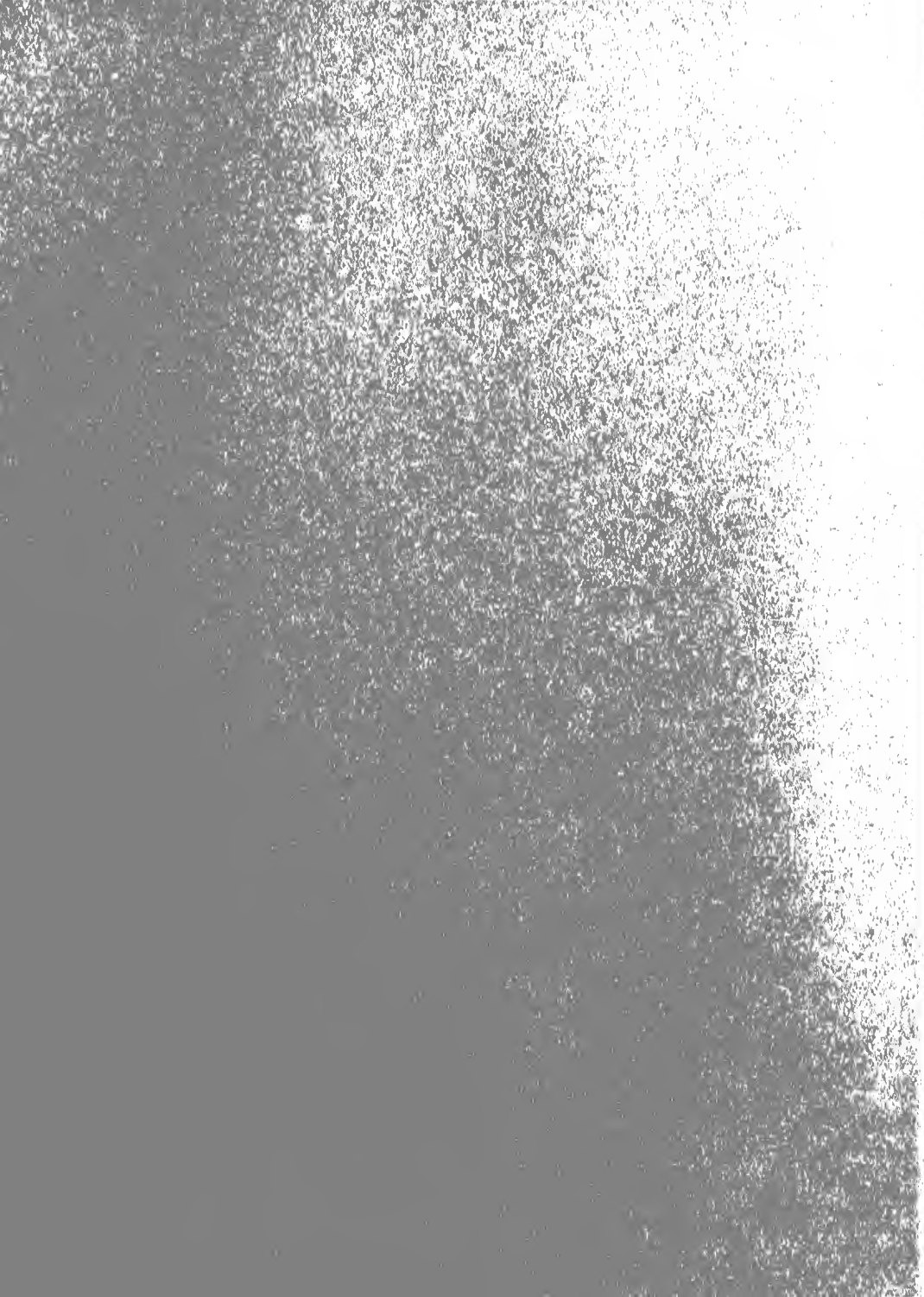
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02699-01 LENP
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Roles of Gangliosides in Neuronal and myelin function, cell growth and differentiation, and neurotoxicity		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: K.-F.J. Chan Senior Staff Fellow LENP NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Experimental Neuropathology		
SECTION Neurotoxicology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.6	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           Gangliosides, a class of sialic acid-containing glycosphingolipids ubiquitous in eukaryotic cells, are highly enriched in the membrane components of the central nervous system (CNS), particularly the nerve terminals. Although these glycolipids have been implicated in the regulation of neurotransmission, cell growth and differentiation, and are involved in the specific interaction with a variety of biological modulators including neurotoxins, interferons, opiates, lymphokines, and glycoprotein hormones, the exact functional role of gangliosides is not known. One goal of this project is to elucidate the basic mechanisms through which biochemical signal transduction across the cell membranes may be mediated by gangliosides. Several different areas of research will be pursued, using protein phosphorylation, a major post-translational modification involved in the regulation of numerous biological processes, as a tool. (1) A novel ganglioside-dependent protein kinase is to be purified to homogeneity from both CNS synaptosomes and myelin. Its physicochemical properties and possible roles in neurotransmission will be characterized and assessed, in conjunction with immunocytochemical studies at the ultrastructural level during development. (2) The molecular mechanism by which gangliosides activate protein kinase C, a pivotal enzyme in numerous metabolic pathways, will be studied together with intracellular distribution of this kinase. These studies should provide further insight on the function of gangliosides and protein kinase C in the nervous system. (3) Involvement of gangliosides and protein phosphorylation in cell growth and differentiation such as myelination and demyelination, synapse formation, oncogenesis and aging will be investigated. (4) The mode of action of neurotoxins, especially those which are known to interact with gangliosides, will be analyzed. New information derived from these investigations should provide better understanding of neuronal and myelin function at both the molecular and cellular levels.         </p>		



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02525-05 LENP																												
PERIOD COVERED October 1, 1985 through September 30, 1986																														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Exocytosis Modelling: Kinetics of Membrane Aggregation and Fusion</b>																														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: S.J. Morris</td> <td style="width: 33%;">Guest Worker</td> <td style="width: 33%;">LENP</td> <td style="width: 33%;">NINCDS</td> </tr> <tr> <td>Others: P.D. Smith</td> <td>Visiting Scientist</td> <td>BEIB</td> <td>DRS</td> </tr> <tr> <td>C.C. Gibson</td> <td>Electronics Engineer</td> <td>BEIB</td> <td>DRS</td> </tr> <tr> <td>D. Bradley</td> <td>Chemist</td> <td>LENP</td> <td>NINCDS</td> </tr> <tr> <td>R. Blumenthal</td> <td>Section Chief</td> <td>LTB</td> <td>NCI</td> </tr> <tr> <td>A. Walter</td> <td>Staff Fellow</td> <td>LTB</td> <td>NCI</td> </tr> <tr> <td>D.L. Siegel</td> <td>Guest Worker</td> <td colspan="2">Proctor and Gamble, Cincinnati, OH</td> </tr> </table>			PI: S.J. Morris	Guest Worker	LENP	NINCDS	Others: P.D. Smith	Visiting Scientist	BEIB	DRS	C.C. Gibson	Electronics Engineer	BEIB	DRS	D. Bradley	Chemist	LENP	NINCDS	R. Blumenthal	Section Chief	LTB	NCI	A. Walter	Staff Fellow	LTB	NCI	D.L. Siegel	Guest Worker	Proctor and Gamble, Cincinnati, OH	
PI: S.J. Morris	Guest Worker	LENP	NINCDS																											
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A. Walter	Staff Fellow	LTB	NCI																											
D.L. Siegel	Guest Worker	Proctor and Gamble, Cincinnati, OH																												
COOPERATING UNITS (if any) Proctor and Gamble, Inc., Cincinnati, OH																														
LAB/BRANCH Laboratory of Experimental Neuropathology																														
SECTION Neurotoxicology Section																														
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																														
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.9	OTHER: 0.1																												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Neurotransmitter and neuromodulator release</u> takes place by <u>exocytosis</u>; the influx of <u>calcium</u> into the cell or nerve terminal triggers the <u>fusion</u> of the <u>storage granule</u> with the <u>cell plasma membrane</u>. The membrane fusion events can be modeled by studying the fusion of artificial or biological membranes with each other. A new multichannel, computer controlled stopped-flow rapid mixing spectrometer has been constructed to study the kinetics of these reactions. Our previous <u>stopped-flow mixing</u> studies have shown that the <u>kinetics</u> of both aggregation and fusion of small vesicular structures (artificial lipid vesicles, neurotransmitter storage granules, etc) follow second order kinetics with fusion being aggregation rate limited.         </p> <p>           Our original stopped-flow assay for membrane fusion, based on <u>resonance energy transfer</u> between <u>fluorescent phospholipids</u> was subject to artifacts arising from the interactions of NBD-labelled phosphatidylethanolamine with the fusion catalysts such as <math>Ca^{2+}</math> interacting directly with the NBD probe and changing its quantum yield. We have developed a new assay, based on changes in the fluorescence of pyrene-labelled phosphatidylcholine. This assay examines the ratio of pyrene excimer/monomer fluorescence as a function of time after mixing. This ratio is independent of calcium concentration and is linearly dependent upon the distance between pyrene fluorophores, thus solving these problems and additionally allowing easy calculation of expected amplitude changes as the vesicles fuse together. This assay has been applied to the Mg-promoted fusion of artificial vesicles as well as protein-catalyzed fusion.         </p>																														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02264-10 LENP																				
PERIOD COVERED October 1, 1985 through September 30, 1986																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Animal Models of Neurological Disease																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: S. M. Anderson</td> <td style="width: 20%;">Guest Worker</td> <td style="width: 20%;">LENP</td> <td style="width: 20%;">NINCDS</td> </tr> <tr> <td>Others: R. Weir</td> <td>Guest Worker</td> <td>LENP</td> <td>NINCDS</td> </tr> <tr> <td>J.W. Daly</td> <td>Chief</td> <td>LBC</td> <td>NIADDK</td> </tr> <tr> <td>C.T. Hansen</td> <td>Animal Geneticist</td> <td>ACRC</td> <td>DRS</td> </tr> <tr> <td>J.T. Petras</td> <td>Neuroanatomist</td> <td>DNP</td> <td>WRAIR/WRAMC</td> </tr> </table>			PI: S. M. Anderson	Guest Worker	LENP	NINCDS	Others: R. Weir	Guest Worker	LENP	NINCDS	J.W. Daly	Chief	LBC	NIADDK	C.T. Hansen	Animal Geneticist	ACRC	DRS	J.T. Petras	Neuroanatomist	DNP	WRAIR/WRAMC
PI: S. M. Anderson	Guest Worker	LENP	NINCDS																			
Others: R. Weir	Guest Worker	LENP	NINCDS																			
J.W. Daly	Chief	LBC	NIADDK																			
C.T. Hansen	Animal Geneticist	ACRC	DRS																			
J.T. Petras	Neuroanatomist	DNP	WRAIR/WRAMC																			
COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIADDK; Animal Genetic Resource, Division of Research Services; Neuroanatomy Branch, Walter Reed Army Institute of Research, Walter Reed Army Medical Center																						
LAB/BRANCH Laboratory of Experimental Neuropathology																						
SECTION Neurotoxicology Section																						
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																						
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0																				
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																						
SUMMARY OF WORK (Use stendera unreduced type. Do not exceed the space provided.) <p>             The purpose of this project is the investigation of basic mechanisms associated with naturally occurring or artificially <u>neurotoxin-induced</u> neurological disease through the use of animal models and <u>in vitro</u> experiments. Interactions of various <u>neuroactive drugs</u> and <u>neurotoxins</u> with neurotransmitters in the central nervous system have provided the focus for combined <u>behavioral</u> and <u>neurochemical</u> studies emphasizing basic mechanisms of action of proposed neurotoxins. Two major interests of this project are: (A) to define populations of individuals that may be at increased risk to neurological disease resulting from exposure to neurotoxins and (B) to use naturally occurring variability in central nervous system function, anatomy and/or neurochemistry, to elucidate mechanisms of actions of neurotoxins. Several different projects have been investigated this year. (1) Interactions of the <u>artificial food color</u>, erythrosin B, with neuronal membranes and neurotransmission have been studied. Erythrosin B has been demonstrated, by several different criteria, to be a potent inhibitor of ATPase activity in brain and other tissues. Its inhibitory potency can be enhanced <u>in vitro</u> by exposing the tissue-erythrosin B complex to light. Our most recent data suggest that the light-enhanced inhibitory actions of erythrosin B may be a general phenomenon affecting a variety of excitable membranes but its dark phase inhibition may be specific to ATPases. (2) We have been investigating an apparently new <u>neurological mutation</u> in rats discovered in our animal breeding unit. Animals display a form of ataxia which appears to be inherited in the pattern characteristic of single gene autosomal traits. Preliminary neuroanatomical studies have discovered some axonal degeneration in the cortical fugal and limbic systems. (3) For several years we have been studying the effects of convulsants and <u>anticonvulsants</u> on <u>adenosine receptors</u> in rat brain. Carbamazepine, a clinically used anticonvulsant, was found to have a major inhibitory action on brain adenosine receptors. We have been unable, however, to classify it as either a pure agonist or antagonist at A<sub>1</sub> adenosine receptors.           </p>																						





ANNUAL REPORT

October 1, 1985 through September 30, 1986

Laboratory of Molecular Biology  
National Institute of Neurological and Communicative  
Disorders and Stroke

TABLE OF CONTENTS

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Control Mechanisms and Differentiation Z01 NS 01244-22 LMB	8
Cellular Responses to Hormones and Neurotransmitters Z01 NS 02365-08 LMB	9



Annual Report  
October 1, 1985 through September 30, 1986  
Laboratory of Molecular Biology  
National Institute of Neurological and Communicative  
Disorders and Stroke

Ernst Freese, Ph.D., Chief

The work in the Laboratory (of Molecular Biology) is now largely concerned with the control and interaction of mammalian neural cells. Only that microbial work is continued which has direct significance for mammalian cell studies or is of fundamental importance to genetic control mechanisms.

1. Isolation, characterization, and control of genes involved in the synthesis and degradation of excitatory and inhibitory amino acids. The maintenance of homeostatic levels of excitatory amino acids (glutamate, aspartate, and their analogs) and of inhibitory amino acids ( $\gamma$ -aminobutyric acid = GABA) is essential for the proper functioning of the brain. Important for this control are the correct levels of enzymes, transport mechanisms, etc. The enzymes apparently are partitioned according to their major function between neurons [e.g., glutaminase (GA) and glutamate decarboxylase (GAD)] and astrocytes [e.g., glutamine synthetase (GS), glutamate dehydrogenase (GDH)]. Inability to maintain the proper equilibrium can result from a change in the blood or oxygen supply, from the presence of toxins or from abnormal genetic factors. Such deviations are apparently responsible for hepatic encephalopathy (hyperammonemia) and hypoglycemic coma, neural necrosis following myocardial infarctions, brain edema resulting from trauma or stroke, and epilepsy and or neurodegenerative disorders such as oligopontocerebellar atrophy. A minor imbalance between excitation and inhibition seems to be responsible for mood alterations that are frequently treated by drugs (e.g., tranquilizers) that reduce the excitatory effect of glutamate or increase the inhibitory effect of GABA. Our objective is to investigate the regulation of genes encoding proteins important in the synthesis and degradation of glutamine, glutamate, and GABA. We are using rats and their genes as a model system and human cell lines and genes to determine the similarities between the two mammalian systems. We also intend to investigate epileptic foci excised from patients.

Using antibodies, we have so far isolated human and rat genes for GDH and the rat gene for GA. We also obtained cDNA probes for GAD from cat and for GS from hamster and used them to isolate clones of rat and human homologs. We have sequenced the cDNA of

GDH and shown that it encodes at least 40% of the human enzyme. Employing the different cDNA clones to generate highly radioactive cRNA, we have probed the mRNA of different brain regions, and found that the GDH/GA ratio was high in some and low in other areas. In situ hybridization to determine the specific mRNA synthesis in more detail is under way. Using the cRNAs, we detected transcripts of about 3.6 kb for GDH and 5.4 kb for GA in both human and rat brain. Whereas the brain had only one mRNA for both enzymes, liver had no GA and two different GDH mRNAs. The latter could imply either a different extent of RNA processing in the two organs or it could relate to the observation by other groups that liver seems to contain two GDHs. If each of the two enzymes is translated from a different RNA, the two cRNAs could be transcribed from two genes related by evolution or they could result from different splicing of the same genomic RNA. The amount of GA mRNA in normal kidney is similar to that in brain and about 5 fold increased during acidosis. Altered levels of GDH, GA, and GS mRNA were also found in rats made encephalopathic by thioacetamide treatment.

We have used the cRNA for glutamine synthetase to show that in rat C-6 glioma cells dexamethasone, a synthetic glucocorticoid, increases the amount of mRNA (size 3 kb) 4-fold while the specific activity of the enzyme increases 2.5-fold. Thus dexamethasone acts at the transcriptional level. Interestingly, the mRNA was much more abundant in adult rat brain than in C-6 cells.

2. Stimulation of cGMP production by excitatory amino acids and its inhibition by tricyclic antidepressants. Excitatory amino acids such as glutamate bind to specific cell surface receptors in cultured neurons and thereby open ion channels and stimulate intracellular cGMP production. This stimulation depends on the presence of extracellular calcium and can be mimicked by the depolarizing agent veratridine, which opens voltage-dependent sodium channels. We have also shown that the presence of the calcium ionophore A23187 stimulates cGMP production in a calcium-dependent manner. Our interpretation is that the increased intracellular calcium whose influx is enabled by these agents stimulates guanylate cyclase, possibly mediated by calmodulin. However, we must also consider the possibility that the glutamate receptor is coupled to guanylate cyclase via membrane components resembling the transducing proteins found in other systems. Interestingly, the stimulation of cGMP synthesis depends on the age of the culture following its establishment from the cerebellum.

We have also found that the tricyclic antidepressant imipramine, which we have shown to accumulate in neurons and astrocytes, inhibits glutamate-stimulated cGMP synthesis. Imipramine accumulates in intracellular vesicles, especially in



dense lysosomes. In C6 glioma cells, we have shown that imipramine causes down-regulation of  $\beta$ -adrenergic receptors within 24 hours, mimicking the clinical response to long-term administration of tricyclic antidepressants. This down-regulation is not due to a direct action of imipramine on  $\beta$ -adrenergic receptors, but seems to result from interference with receptor recycling because the drug accumulates in endocytotic vesicles.

3. Control of the expression of glial fibrillary acidic protein (GFAP) in glioma cells. GFAP is the only known astrocyte-specific protein and is therefore critical in the decision whether brain tumors are of astrocyte origin or derived from other cell types. A previous report (Duffy, 1983) claimed that GFAP expression is lost during tumor progression so that malignant tumors usually do not show any GFAP. We have examined this in ten glioma cell lines derived from both benign and malignant tumors and indeed found that by immunofluorescence only five of the cultures were positive for GFAP. However, when we isolated the proteins from these cultures and ran an immunoblot, all ten cultures showed a positive GFAP reaction which was of the same strength in each case. This shows that, at least for GFAP, immunofluorescence staining of tissues or cells is not a reliable method to detect the presence of GFAP. Apparently, some other factor is necessary to enable the assembly of GFA protein. We also used the gene for GFAP to isolate radioactive cRNA and showed that the mRNA of all ten cultures contained GFAP message. Since several of these cultures were malignant, we conclude that there is no correlation between malignancy and the loss of GFAP as long as one examines astrocytomas directly or uses cells derived from them and maintained in culture for at most fifteen passages.

4. Characterization of nuclear and membrane proteins in brain cells. Since astrocytes can be identified so far only by the GFA protein, we have attempted to identify additional brain/astrocyte specific protein markers. In plasma membranes we found about 400 polypeptides ( $pI = 4.6$  to  $6.8$ ;  $MW = 10,000$  to  $90,000$ ). Two polypeptides, MP1 and MP2 comprised about ten percent of the total. They were initially isolated from astrocytes and were then also found in C-6 cells and granular neurons but not in kidney or liver. Persistent efforts to raise antisera against them have failed, which indicates that they are important proteins against which mammals develop tolerance. Their general properties make them similar to an intensely investigated protein found in nerve growth cones; we are determining the properties of the two proteins and their relationship to growth cone proteins.

Since we had developed methods to isolate nuclei and their matrices, we investigated the corresponding proteins in several cell types. Some nuclear proteins were present only in neurons

and others only in astrocytes. Cells in culture showed generally more types of proteins than cells isolated directly from adult rat brains presumably because the cells in culture are proliferating. For one of the nuclear matrix proteins, a monoclonal antibody was used to isolate the corresponding cDNA. This and the gene for lamin (a structural protein) are the only genes for nuclear matrix proteins which have been cloned. Our cDNA has been partially sequenced; about 900 base pairs of it suffice to code for the protein. The role of this protein in DNA binding or during mitosis is being investigated.

The concentration of several nuclear proteins increased in astrocytes cultured in the presence of high levels of ethanol or ammonia, conditions known to cause neurological disorders. The properties of one protein are similar to a stress protein found in eucaryotes, which indicates that conditions of hyperammonemia are regarded by the cells as general stress.

5. Control of meiosis and glycogen synthesis by GTP and cAMP. We had previously shown that the initiation of yeast meiosis and sporulation, i.e., of differentiation, is specifically caused by the decrease of guanine nucleotides. Another group had claimed that decreased cAMP may also be necessary for the initiation process. We have used conditions under which guanine nucleotides decrease while the cAMP decrease was completely avoided, by the addition of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), and yet we found excellent sporulation. In similar experiments, we have shown that cAMP has nothing to do with catabolite repression because cells growing in glucose had the same amount of cAMP as cells growing in acetate as sole carbon source. We also crossed the cancer mutation RAS<sup>val</sup> into our guanine mutant and showed that the RAS<sup>val</sup> mutation was dominant in preventing sporulation during both deprivation and guanine deprivation. The glycogen increase normally found during meiosis induction was also prevented by the RAS<sup>val</sup> mutation. Thus, the mutation RAS<sup>val</sup> effects not only the growth of cells but also their differentiation at least in yeast.
6. Sequence comparison and control of the developmental gene for glucose dehydrogenase. During the differentiation (sporulation) of Bacillus subtilis several new proteins are synthesized which are not found during vegetative growth. One of these proteins is the enzyme for glucose dehydrogenase which is made only inside a special cell compartment (forespore). To understand why this enzyme is produced only during differentiation, we have isolated the gene and sequenced it. We have determined that a 0.5 kb fragment preceding the structural gene for the enzyme controls the expression of the enzyme during differentiation. To our surprise, removal of this 0.5 kb region enabled constitutive expression of

the enzyme already during vegetative growth. Conceivably, the expression is prevented by a stem/loop area located in the 0.5 kb region. When this stem/loop is present, it may have to be melted by some mechanism which may be switched on only during sporulation. We are using in situ mutagenesis and insertion of the 0.5 kb region into a plasmid containing a promoterless chloramphenicol transacetylase gene to determine the initiation mechanism.

We have compared the nucleotide sequence of the structural gene for glucose dehydrogenase gene in E. subtilis with the known amino acid sequence of the Bacillus megaterium enzyme. When we determined the types and number of mutations needed to convert one gene into the other, we found that 23 transitions, 30 transversions, one inversion, and three insertions/deletions were needed as a minimum. Interestingly, no frameshift occurred, which indicates that the enzyme was essential for the survival of the organism throughout evolution and any mutation changing the reading frame was lethal. In spite of these mutations, the regions essential for the binding of the four subunits and of NADH are essentially unchanged. In one subunit binding area one gene has two amino acids less than the other; we are now determining by in situ hybridization whether this change is unimportant or whether it had to be compensated by amino acid changes in other subunit binding areas. We are also checking whether the subunit binding properties can be modified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02677-02 LMB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Differentiation and Regulation of Gene Expression in Glial Cells</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) L. Vitkovic, Senior Staff Fellow, LMB, NINCDS (P.I.) C. Banner, Senior Staff Fellow, LMB, NINCDS E. Freese, Chief, LMB, NINCDS R. Lipsky, Staff Fellow, LMB, NINCDS J. Thomas, Staff Fellow, LMB, NINCDS M. Dohadwala, Visiting Fellow, LMB, NINCDS K. Lampel, Senior Staff Fellow, LMB, NINCDS *		
COOPERATING UNITS (if any) Institute of Neurology, University of London (M. Noble) Section on Structural Plasticity, LMB, NINCDS (M. Brightman) Laboratory of Oto-laryngology, NINCDS (R. Wenthold) Dept. of Biochemistry, University of Pittsburgh (N. Curthoys)		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION Developmental Biology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 6.7	PROFESSIONAL: 5.7	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>Our objective is to investigate the regulation of genes encoding proteins important in the function of <u>astrocytes</u> and in <u>neuron-glial interaction</u>. To this end, we have screened human and rat brain expression libraries for cDNAs encoding enzymes of the GABA-glutamate-glutamine cycle. By screening with antibodies, we have isolated cDNA clones for human glutamate dehydrogenase (GDH) and rat glutaminase (GA). Using these cDNAs as probes and cDNAs for glutamine synthetase (GS) and glutamate decarboxylase (GAD), we have compared mRNA levels for these enzymes in various brain regions in liver, and in kidney. We have established that GS mRNA is induced in C6 glioma cells by dexamethasone, a glucocorticoid. In a rat model for hepatic encephalopathy, we have shown that brain mRNA levels for GS, CDS, and GA are altered. Using the gene for <u>glial fibrillary acidic protein</u> (GFAP) as a marker, we have shown that <u>immunofluorescence</u> is much less reliable than <u>immunoblots</u> to determine the presence of GFAP in human gliomas. One cell line was developed which shows 20 fold induction of GFAP synthesis after addition of <u>mycophenolic acid</u>.</p> <p>Several <u>nuclear proteins</u> of neurons and astrocytes were identified, two of which significantly increased in amount when astrocytes were cultured under conditions that <u>in vivo</u> cause <u>neurological disorders</u> (0.4 M ethanol or 3.8 mM ammonia). Two abundant plasma <u>membrane proteins</u> present in both neurons and astrocytes but absent in membranes of kidney and liver cells were identified and purified.</p> <p>-----</p> <p>*Continued:          K. Mearow, Visiting Fellow, LMB, NINCDS          H. Steisslinger, Visiting Fellow, LMB, NINCDS          S. Silverman, Senior Staff Fellow, LMB, NINCDS</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02680-02 LMB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nucleotide Sequence and Control of the GlcDH Operon

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

K. A. Lampel, Senior Staff Fellow, LMB, NINCDS (P.I.)

E. Freese, Chief, LMB, NINCDS

Y. Nakatani, Visiting Fellow, LMB, NINCDS

COOPERATING UNITS (if any)

Dr. R. F. Ramaley, University of Nebraska Medical Center, Omaha, Nebraska

Prof. P. Fortnagel, Inst. fur Allgemeine Botanik, West Germany

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.6

PROFESSIONAL:

2.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bacillus subtilis undergoes differentiation (sporulation) when it encounters nutrient deprivation. Many new proteins are synthesized only during sporulation. The mechanisms by which this bacterium controls the expression of these developmentally regulated genes are not known. We are currently investigating the mechanism(s) controlling one sporulation specific gene, the glucose dehydrogenase gene (gdh). This gene and an uncharacterized gene that precedes gdh comprise an operon that is expressed only during sporulation. The nucleotide sequence and ribosome binding sites have been determined. The promoter is located within a 0.5 kb PvuI region and has been narrowed to be between AluI and PvuI sites. No nucleotide sequence in this fragment matches any of the known promoter sequences of the B. subtilis RNA polymerases. DNA fragments that contain the putative promoter sequence of the gdh operon were inserted in front of plasmid-borne promoter-less genes to determine which sequences are necessary for sporulation control of gdh. A comparison between the B. subtilis DNA sequence and the B. megaterium amino acid sequence showed how many and what type of mutations occurred during the evolution of gdh.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 01244-22 LMB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Control Mechanisms and Differentiation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  E. Freese, Chief, Laboratory of Molecular Biology, NINCDS (P.I.) Z. Olempska-Beer, Visiting Associate, LMB, NINCDS S. Silverman, Senior Staff Fellow, LMB, NINCDS		
COOPERATING UNITS (if any)  None		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION Developmental Biology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.9	PROFESSIONAL: 1.9	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             This report is the last one concerning work on the induction of mitosis and meiosis in the yeast <i>Saccharomyces cerevisiae</i> and the characterization of nuclear proteins in yeast and mouse cells. Previously, we had found that meiosis is initiated by the specific decrease of GTP. We have now shown that this initiation does not require a change in the concentration of cAMP and that GTP deprivation also causes an increase in the synthesis of glycogen. cAMP also does not mediate catabolite repression. But the presence of a carbon source is required for the normal completion of spores. Since the RAS<sup>val</sup> gene prevents sporulation of yeast during nitrogen starvation, we have constructed a <u>gua</u> RAS<sup>val</sup> double mutant; it cannot sporulate or produce glycogen during guanine deprivation which shows that the RAS<sup>val</sup> mutation is always dominant. We have also characterized nuclear matrix proteins and isolated the cDNA gene for one of them from a mouse brain cDNA library.           </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02365-08 LMB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Responses to Hormones and Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R. C. Henneberry, Chief, Molecular Neurobiology Section, Laboratory of  
Molecular Biology, NINCDS (P.I.)

P. G. Lysko, Senior Staff Fellow, LMB, NINCDS

A. Novelli, Visiting Fellow, LMB, NINCDS

M. D. Collado, Visiting Fellow, LMB, NINCDS

## COOPERATING UNITS (if any)

Myelin and Brain Development Section, Developmental and Metabolic Neurology  
Branch, NINCDS

## LAB/BRANCH

Laboratory of Molecular Biology

## SECTION

Molecular Neurobiology Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

4.0

## PROFESSIONAL:

3.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

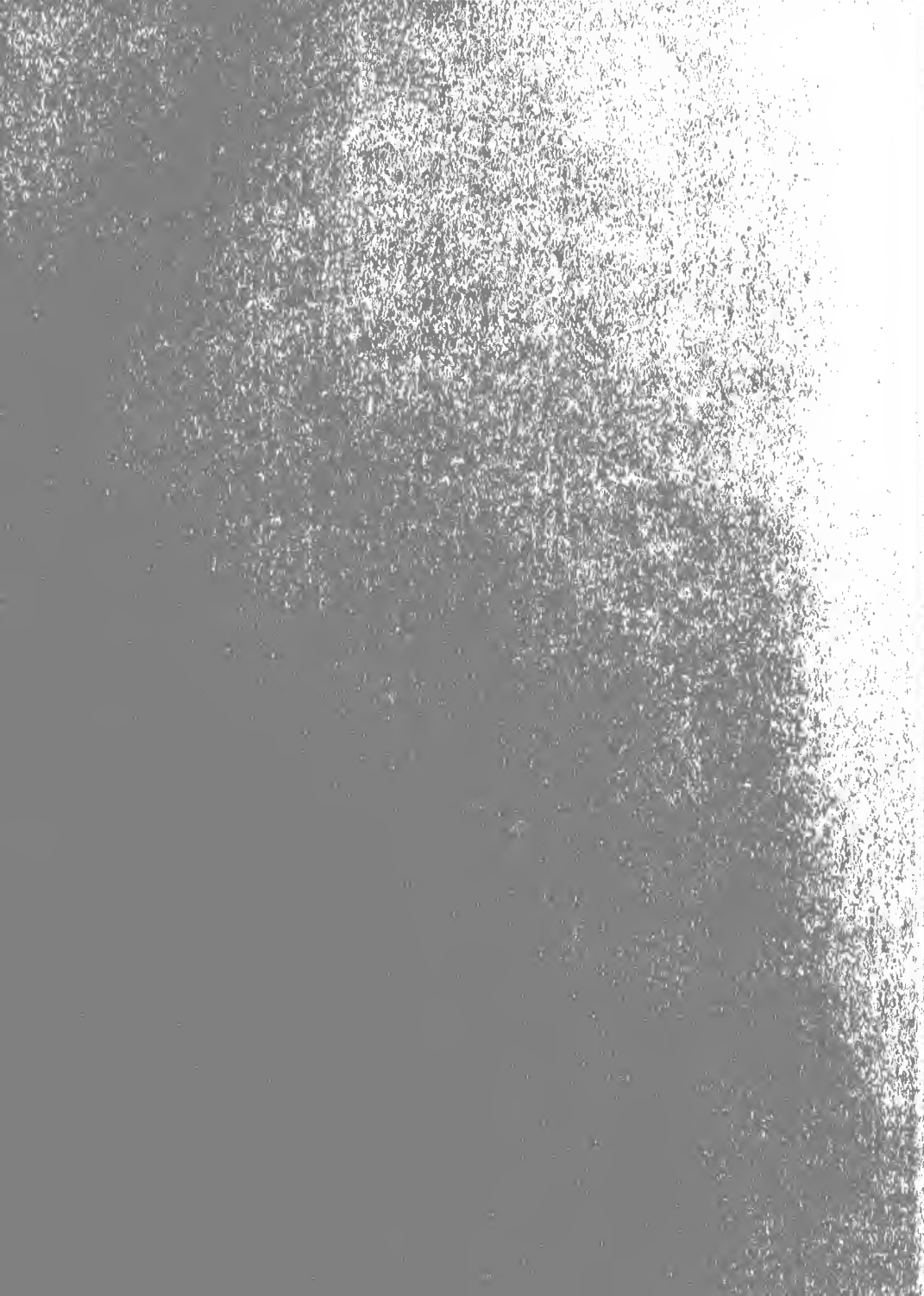
Our objective is to elucidate how cells derived from the mammalian brain perceive and respond to the signals in their environment. We have studied the effects of hormones and neurotransmitters which transmit information across the plasma membrane while binding to and remaining at cell-surface receptors of neurons and astrocytes. In rat astrocytoma cells, we have shown that certain neurotransmitter-stimulated, receptor-mediated increases in cyclic AMP synthesis are modulated by tricyclic antidepressants such as imipramine after the drugs are transported into cells and accumulated in subcellular organelles. We have shown that growth of astrocytoma cells in the presence of imipramine causes down-regulation of beta-adrenergic receptors, a phenomenon which mimics the clinical response to long-term administration of imipramine. Our results suggest that imipramine may cause down-regulation of these receptors by accumulating in endocytic vesicles and impairing receptor recycling. Using primary cultures highly enriched for a particular type of neuron, we have found that the ability to respond to stimulation of cyclic GMP production by excitatory amino acids is related to the developmental stage of the neurons. The stimulation of cGMP production correlates with the stimulation of neuro-transmitter release from these neurons. Furthermore, stimulation of cyclic GMP production by excitatory amino acids and by depolarizing agents is calcium dependent and is inhibited by imipramine. Our results implicate calcium in the activation of guanylate cyclase, the biosynthetic enzyme for cyclic GMP, and suggest a biochemical basis for the manifold actions of tricyclic antidepressants. We have found that imipramine and related compounds are also concentrated in intracellular vesicles in cultured neurons, and we are presently investigating the possible role of tricyclic antidepressants in the depletion of neurotransmitters in secretory vesicles.

9 - LMB/IRP









# ANNUAL REPORT

October 1, 1985 through September 30, 1986

## Laboratory of Molecular Genetics

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT  
October 1, 1985 through September 30, 1986  
Laboratory of Molecular Genetics  
National Institute of Neurological and Communicative  
Disorders and Stroke

Robert A. Lazzarini, Ph.D., Chief

A major strength of the Laboratory of Molecular Genetics is its breadth of expertise -- from cellular biology to molecular biology. It has allowed the laboratory to study biological phenomena simultaneously at both the cellular and molecular levels and to reap the advantages of the cross-stimulation and exchange between the approaches. During the past five years, we have capitalized on this strength by setting up parallel research programs directed at the same or closely related phenomena -- one operating at the molecular level, the other at the cellular. Thus, we have established programs concerning the "Regulation of Myelin Synthesis" (Lazzarini, PI) and the "Biology of Myelin-Forming Cells In Vitro and In Vivo" (Dubois-Dalcq, PI). The parallel virus programs concern the "Regulation of Viral Nucleic Acid Synthesis in Animal Cells" (Schubert, PI) and the ultrastructural analyses of the "Assembly of Enveloped RNA Viruses" (Dubois-Dalcq, PI). This theme was expanded upon this year by creating two new programs each of which has a molecular and a cellular component. In the "Degenerative Processes of the Nervous System" program, we are exploring the structure and origin of the amyloid and neurofibrillary tangles that accumulate in the brains of patients with Alzheimer's disease. Initially, we wish to find out whether they are derived from neurofilaments, the intermediate filaments of neurons. In the "Biology of Mammalian Homeo Domain Proteins" program, the function of several of these master regulatory proteins that control embryonic development of the nervous system are being studied. We are also studying the organization of the genes encoding the proteins and determining the tissue and cell type in which these genes are expressed and at what time during development the genes are active.

The Myelin Programs:

We have established a cell culture system for the study of the sequential events leading to the differentiation of the oligodendrocytes, the myelin-forming cells of the CNS. In this system, precursor cells isolated from embryonic rat brain or optic nerve develop through a succession of stages that can be monitored using fluorescently tagged antibodies which recognize the specific proteins acquired during the development of the mature oligodendrocyte. Only late in the developmental program do the oligodendrocytes synthesize the proteins found in myelin. In brain-derived oligodendrocytes, myelin basic protein (MBP) and myelin associated glycoprotein (MAG) first emerge between the fifth and the seventh day of culture, followed by proteolipid protein (PLP) on the eighth to ninth day. Each protein shows a specific cytoplasmic localization. MBP and PLP are seen in the extremities of the cell processes only after an additional five days in culture. At this time, numerous membrane and myelin whorls accumulate along the cultured oligodendrocyte surface. The sequential emergence, cytoplasmic location, and peak of expression of the three myelin proteins in vitro follow a pattern

similar to that observed in vivo. This program is, therefore, independent of continuous neuronal influences. These cultures which appear to mimic precisely the developmental changes in vivo, provide a convenient system for the study of factors regulating and affecting the oligodendrocyte development. Consequently, this system was used to search for mitogens and growth factors that specifically affect the glial progenitor cells. In addition, a transformed rat CNS cell was isolated that elaborates a potent mitogen for the oligodendrocyte progenitor cells which we are currently characterizing. Similarly, we are studying the interaction between the oligodendrocyte precursor and rat sensory neurons. It appears that sensory neurons might also provide mitogenic signals to the glial precursors.

The sequential appearance of the myelin specific proteins and the effects of mitogens, must ultimately be explained at the level of the gene encoding these proteins. To this end, we cDNA cloned the myelin basic protein and proteolipid protein mRNAs and used these clones to identify and characterize the genes encoding these proteins. Having studied the organization of these genes and having completely sequenced them, our results demonstrate conclusively that the multiple forms of MBP which are found in both man and mouse originate by a mechanism of alternative splicing of the nascent gene transcript. Alternative splicing appears to be an important way to increase the diversity of related proteins. This mechanism has been preserved in such unrelated species as man and mouse. Furthermore, the fact that many other myelin proteins occur in multiple forms suggests that this process is widely used in the oligodendrocyte.

In an effort to understand at the molecular level how MBP and PLP genes are controlled, we examined and characterized two mutant strains of mice which are unable to synthesize myelin. The Shiverer mouse was found to suffer from a massive deletion in the myelin basic protein gene. The Jimpy mouse was found to suffer from a small deletion in the proteolipid gene. Interestingly, neither mutant mouse was able to synthesize normal amounts of any of the myelin specific proteins, suggesting that there is a coordinate control of these genes, and alterations of one gene has a pleiotropic effect on the others. We have now initiated a program in which we will attempt to introduce the normal wild-type gene into these mice in order to correct their genetic defect.

### The Viral Programs:

The elucidation of the mechanisms controlling the synthesis, processing and transport of viral components will help us understand the defective viral assembly that has been implicated in a number of neurological diseases. To study the biosynthesis of viral macromolecular components, we prepared monoclonal antibodies directed at different epitopes on several measles and vesicular stomatitis virus polypeptides. These immune reagents were purified, concentrated and microinjected into cells before and after viral infection in order to arrest viral assembly at different stages. By combining this approach with EM immunolabeling, we determined how the nucleocapsid interacts with the viral glycoprotein which is embedded in the cellular membrane and how the M protein may induce nucleocapsid coiling and viral assembly. By employing temperature-sensitive mutants in the M protein, we can inactivate the M protein by simply raising the temperature to 40°C. Parallel to these ultrastructural and immune EM studies are the molecular studies which have shown that the

relative proportions of the viral protein in the infected cell are crucial to a successful infection. In particular, too high a concentration of the viral RNA polymerase leads to an aborted infection of the cell. This unsuspected phenomenon offers a potential explanation for the phenomenon of heterotypic autointerference by some defective interfering virus particles. It is interesting that only overexpression of the viral RNA polymerase and not two other viral proteins leads to the arrest of the infection. Through the use of recombinant DNA and proteins synthesized from recombinant cloned genes, we are attempting to reconstruct the interaction of several viral proteins in vitro. Such an in vitro reconstruction will afford us with a system that can easily be studied and manipulated. To this end, we cloned the five genes of the vesicular stomatitis virus and are attempting to obtain biochemical amounts of the proteins encoded by the genes through the use of expression vectors. We will attempt to reproduce in vitro what we see at the cellular level with the electron microscope and fluorescent antibodies.

#### Degenerative Diseases Program:

In the "Degenerative Processes of the Nervous System" program, we are exploring the possibility that the amyloid and neurofibrillary tangles found in the brains of Alzheimer's patients are derived from neurofilaments. We isolated cDNA and genomic clones for the small and mid-size neurofilament proteins of humans. These cDNAs have been completely sequenced and the amino acid sequence of neurofilaments determined. Genomic clones were sequenced to define the organization of the gene. The predicted amino acid sequence of the mid-size neurofilament, which was not known heretofore, exhibits a highly charged, highly  $\alpha$ -helical tail region bearing six tandem repeats of a 13 amino acid sequence. We believe this repeated region is a hinge region and a major site of phosphorylation of the protein. Phosphorylation at the hinge region would be expected to alter the conformation and the macromolecular interactions of the protein. In comparing the amino acid sequence for the mid-size and the small neurofilament subunits with the sequence of the neurofibrillary tangles and amyloid proteins, we found that there is no homology between these Alzheimer's proteins and the neurofilament proteins, indicating that the Alzheimer's proteins were not derived from the two smaller neurofilament subunits.

#### Neuroembryology Program:

Proteins containing the "homeo box" sequence have been shown in several organisms to function as regulatory proteins which control important aspects of embryonic development. We anticipate that certain "homeo box" proteins must control glial and neuronal differentiation in the central nervous system. In our search for evidence to support this hypothesis, we isolated a cDNA of a "homeo box" containing protein from mouse brain and completely sequenced the clone. This cDNA specifies a protein which appears in the mouse embryo about halfway through the normal gestation. In addition, oligopeptides corresponding to various regions of the protein were synthesized and antibodies against these peptides were raised. We are now using these antibodies to localize the expressed protein and to precisely determine at what time during development, in what tissue and in what part of the cell the protein appears.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02034-14 LMG
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Biology of Myelin-Forming Cells In Vitro and In Vivo</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. Dubois-Dalcq	Section Chief* LMG, NINCDS
Others:	N. Zeller	Senior Staff Fellow LMG, NINCDS
	T. Behar	Microbiologist LMG, NINCDS
	R. Rusten	Biological Lab Technician LMG, NINCDS
*M. Dubois-Dalcq has been on a foreign study assignment at the University College, London during this year.		
COOPERATING UNITS (if any) K.V. Holmes, Dept. of Pathology, USUHS; D. Pleasure, Univ. of Pennsylvania; L. Hudson, Lab. of Molecular Genetics, NINCDS; K. Kristensson, Karolinska Inst., Stockholm; M. Raff, H. Geller, and W. Richardson, Univ. College, London.		
LAB/BRANCH Laboratory of Molecular Genetics		
SECTION Neural and Molecular Ultrastructure Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Myelin sheath is essential to normal conduction in nerves and is altered in multiple sclerosis and Guillain-Barre diseases. Understanding how myelin is formed and repaired requires basic studies of the differentiation of myelin-forming cells both <u>in vitro</u> and <u>in vivo</u>. Within the developing rat central nervous system (CNS), progenitor cells of oligodendrocytes are stimulated to divide by a factor secreted by Type I astrocytes. We are characterizing this mitogenic factor using a transformed cell line derived from rat CNS which produces a potent mitogenic activity for these progenitors. In a microculture system, a single neonatal progenitor cell in contact with a layer of Type I astrocytes produces a clone of oligodendrocytes containing a slowly dividing "adult" type progenitor which may be able to repair myelin in the adult. We are also studying <u>in vitro</u> how defined populations of neurons influence the oligodendrocyte differentiation pathway. To further analyze the factors that control the development of the oligodendrocyte lineage at both the molecular and cellular level, we are using a rat myelin deficient mutant (sex-linked lesion) which fails to synthesize myelin protein gene mRNAs and appears to have a block in early oligodendrocyte differentiation. Finally, we are studying the regeneration of CNS myelin in a demyelinating disease (caused by a virus in mice). We have detected a widespread activation of myelin basic protein gene expression in the normal white matter surrounding the lesion using <u>in situ</u> hybridization.</p> <p>The regulation of expression of major myelin proteins in the CNS (PLP) and peripheral nervous system (PNS) (<math>P_0</math>) is another object of our investigation. We determined which domains of the PLP molecule are exposed to the extracellular space in living oligodendrocytes and compared hybrid Schwannoma cells to normal Schwann cells <u>in vitro</u> to analyze what blocks translation of <math>P_0</math> as well as MBP in the absence of neurons.</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 NS 02528-05 LMG

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Myelin Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. A. Lazzarini	Chief	LMG, NINCDS
Others:	L. Hudson	Senior Staff Fellow	LMG, NINCDS
	J. Kamholz	Medical Staff Fellow	LMG, NINCDS
	C. Puckett	Medical Staff Fellow	LMG, NINCDS
	J. Toffenetti	Senior Staff Fellow	LMG, NINCDS
	W. Jacob	Staff Fellow	LMG, NINCDS
	J. Berndt	Microbiologist	LMG, NINCDS
	H. Engh	Biologist	LMG, NINCDS

COOPERATING UNITS (if any)

Dr. Francesca de Ferra, The Wistar Institute, Philadelphia, PA; Dr. Susan Molineaux, Howard Hughes Medical Institute, New York, NY (both are former employees of the Laboratory of Molecular Genetics).

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Recombinant Genetics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.75

PROFESSIONAL:

2.75

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this umbrella project is the detailed understanding of the developmental program that culminates in myelin synthesis. We are particularly interested in obtaining molecular level information about the control of expression of the myelin basic protein gene and the proteolipid protein gene. To this end, we cDNA cloned and sequenced both human and mouse myelin basic protein mRNA and proteolipid protein mRNA. We used these characterized cDNAs to identify and study the organization of the genes encoding them. Our results demonstrate conclusively that the multiple forms of myelin basic protein which are found in both man and mouse originate from a mechanism of alternative splicing of the nascent gene transcript.

Genes of two mutant mice have also been well characterized. The Shiverer mouse was found to have a massive deletion in the myelin basic protein gene, whereas the Jimpy mouse has a small deletion in the proteolipid protein gene. Consequently, neither mutant mouse is able to synthesize myelin and both mutants suffer from a neurological disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02600-04 LMG
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Assembly of Enveloped RNA Viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: M. Dubois-Dalcq                      Section Chief*                      LMG, NINCDS  Others: C. Jordan                      Staff Fellow                      LMG, NINCDS K. Ono                      Visiting Fellow                      LMG, NINCDS R. Rusten                      Biological Lab Technician                      LMG, NINCDS		
*M. Dubois-Dalcq has been on a foreign study assignment at the University College, London during this year.		
COOPERATING UNITS (if any)  Dr. Manfred Schubert, Molecular Virology Section, LMG		
LAB/BRANCH Laboratory of Molecular Genetics		
SECTION Section on Neural and Molecular Ultrastructure		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.3	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The elucidation of mechanisms of synthesis, processing and transport of viral components at the molecular level will help us to understand problems of defective viral assembly implicated in some neurological diseases. To study the biosynthesis of viral macromolecular components, we prepared monoclonal antibodies reacting with different sites of polypeptides of two negative stranded RNA viruses (vesicular stomatitis virus [VSV] and measles virus), polyclonal antibodies made against synthetic peptides corresponding in sequence to portions of the viral polypeptides, and genes coding for some of the viral polypeptides either cloned into convenient expression vectors or labeled to detect viral mRNAs in cells by <u>in situ</u> hybridization.</p> <p>For studies of viral assembly, high resolution views were obtained from platinum replicas of the outer and inner side of the plasma membrane of cells infected with measles and VSV. Combining this approach with EM immunolabeling techniques for viral proteins, we determined how the nucleocapsid interacts with the viral glycoprotein integrated in the plasma membrane and how the M protein may induce nucleocapsid coiling during viral maturation. Studies with a temperature-sensitive mutant in the M protein of VSV indicate that M protein normal transport to the membrane and normal conformation are necessary for nucleocapsid coiling and viral budding to occur. Cellular fractionation studies and gel analysis show that, at nonpermissive temperatures, abnormal M proteins accumulate around the perinuclear membranes. The function of the nonstructural protein C of measles virus was analyzed in infected cells microinjected with specific antibodies to this protein. C protein which is associated with viral nucleocapsids inside the cell may be regulating the level of transcription of the other viral proteins.</p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02026-14 LMG

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Viral Nucleic Acid Synthesis in Animal Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Schubert	Research Chemist	LMG, NINCDS
-----	-------------	------------------	-------------

Others:	E. Meier	Visiting Fellow	LMG, NINCDS
	G. Harmison, II	Chemist	LMG, NINCDS
	L. Hudson	Senior Staff Fellow	LMG, NINCDS
	E. Boltz	Visiting Fellow	LMG, NINCDS
	P. Brayton	Staff Fellow	LMG, NINCDS
	J. Faw	Student Aid	LMG, NINCDS

## COOPERATING UNITS (if any)

Dr. Harshad Thacore, University of Buffalo, Buffalo, NY; Dr. Sue Emerson, University of Virginia, Charlottesville, VA; Drs. Monique Dubois-Dalcq and Kenzo Ono, Neural and Molecular Ultrastructure Section, LMG.

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Molecular Virology Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

3.5

## PROFESSIONAL:

3.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Despite the medical importance of negative strand RNA viruses, little is known about the essential viral specific mechanisms involved in transcription, replication and viral assembly. The following aspects are being studied using the five cloned genes of the rhabdovirus, vesicular stomatitis virus (VSV):

1. Identification of the multiple functions of the RNA dependent RNA polymerase L through site specific mutagenesis.
2. Localization of the functional domains within the polymerase complex.
3. Identification of the nucleotide sequences required for transcription initiation, polyadenylation, encapsidation, replication, cell killing and viral assembly using a novel, recombinant, defective RNA virus particle.

Towards these goals, we have cloned, sequenced and expressed both polymerase proteins L and NS of VSV in eucaryotic cells. Surprisingly, overexpression of functional polymerase protein L in COS cells specifically arrests a wild-type virus infection. Unlike with L, overexpression of the N or NS proteins does not cause virus arrest. These data suggest that transcription of nonsegmented negative strand viruses is autoregulated by the highly conserved gene order and the sequential and attenuated mode of transcription. L overexpression also arrests VSV of a different serotype which sheds light on the potential mechanisms of heterotypic exclusion between serotypes as well as heterotypic autointerference.

In order to generate a novel, recombinant, defective VSV particle, we constructed a 160 bp "mini VSV genome" from eight overlapping synthetic oligonucleotides. We are planning to transcribe an RNA from this construct with precise VSV terminal sequences, and to encapsidate and to propagate it with the help of parental virus. It is anticipated that all of these studies will identify essential, viral specific mechanisms which can potentially be the target for the treatment or prevention of viral infections of the CNS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02714-01 LMG																								
PERIOD COVERED October 1, 1985 through September 30, 1986																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Degenerative Processes of the Nervous System</b>																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">R. A. Lazzarini</td> <td style="width: 25%;">Chief</td> <td style="width: 20%;">LMG, NINCDS</td> </tr> <tr> <td colspan="4"> </td> </tr> <tr> <td>Others:</td> <td>D. Nelson</td> <td>Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td></td> <td>C. Puckett</td> <td>Medical Staff Fellow</td> <td>LMG, NINCDS</td> </tr> <tr> <td></td> <td>M. Myers</td> <td>Guest Worker</td> <td>LMG, NINCDS</td> </tr> <tr> <td></td> <td>P. Schneidman</td> <td>Medical Staff Fellow</td> <td>LMG, NINCDS</td> </tr> </table>			PI:	R. A. Lazzarini	Chief	LMG, NINCDS					Others:	D. Nelson	Staff Fellow	LMG, NINCDS		C. Puckett	Medical Staff Fellow	LMG, NINCDS		M. Myers	Guest Worker	LMG, NINCDS		P. Schneidman	Medical Staff Fellow	LMG, NINCDS
PI:	R. A. Lazzarini	Chief	LMG, NINCDS																							
Others:	D. Nelson	Staff Fellow	LMG, NINCDS																							
	C. Puckett	Medical Staff Fellow	LMG, NINCDS																							
	M. Myers	Guest Worker	LMG, NINCDS																							
	P. Schneidman	Medical Staff Fellow	LMG, NINCDS																							
COOPERATING UNITS (if any)  Drs. William Schlaepfer and Virginia Lee, Division of Neuropathology, Department of Pathology, University of Pennsylvania School of Medicine.																										
LAB/BRANCH Laboratory of Molecular Genetics																										
SECTION Recombinant Genetics Section																										
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892																										
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0																								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             We synthesized oligopeptides corresponding to the sequences reported for the amyloid core and neurofibrillary tangle proteins found in brains of Alzheimer's patients. Antibody was raised against these peptides. Using these immune reagents, we demonstrated that there are proteins in normal controls which cross-react with the antisera and are probably the precursors of the pathologically accumulated protein.           </p> <p>             We explored the possibility that the amyloid and neurofibrillary tangles are derived from neurofilaments, the intermediate filament of neurons. We isolated cDNA and genomic clones of the small and mid-size neurofilament subunit of humans. These cDNA clones were sequenced to determine the amino acid sequence of the protein. The genomic clones were sequenced to define the organization of the gene and the position of the introns. The predicted protein for the mid-size neurofilament demonstrates a highly charged, highly <math>\alpha</math>-helical tail region with a proline and serine-rich 13 amino acid sequence repeated tandemly six times. The repeat regions appear to allow the otherwise helical protein to bend, indicating that phosphorylation at this site might alter the protein's conformation. The gene structure of the mid-size subunit and small subunit are similar to one another, but different from the general structure found for the other intermediate filaments of the cell. These results support the notion that both subunits of neurofilaments had a common origin. A comparison of the amino acid sequences of the small and mid-size neurofilaments with those found for neurofibrillary tangles and the amyloid protein reveals no homology, indicating that the later were not derived from the former by any degradative procedure.           </p>																										

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02698-01 LMG

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of Mammalian Homeo Domain Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. Odenwald	Microbiologist	LMG, NINCDS
Others:	C. Taylor	Visiting Fellow	LMG, NINCDS
	F. Palmer-Hill	Geneticist	LMG, NINCDS
	B. Jones	Biological Lab Technician	LMG, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Recombinant Genetics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.75

PROFESSIONAL:

1.5

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Homeosis refers to a class of mutations which brings about the replacement of one body part with that of another normally found elsewhere on the animal. Molecular analysis of the Drosophila homeotic regulatory genes has shown that the proteins which they encode contain a highly conserved 60 amino acid domain (the homeo domain). Homeo domain containing proteins are part of the mechanism which controls the diversity of body parts in Drosophila. The significance of the homeo domain has been highlighted by the recent demonstration that homeo domain proteins are found in other metazoans, including mice and humans. These observations suggest the exciting possibility that genes which control the insect body plan may have partial homology to functionally related genes which control the body plans of mammals. By identifying the functional roles these genes play in mouse development, we hope to gain insight into the basic principles which govern mammalian development.

Initiated in January 1986, the first phase of this project for one of the mouse genes is near completion. Utilizing the nucleic acid sequences that encode a Drosophila homeo domain as a tool, we have cloned and sequenced an homologous mouse gene and its corresponding cDNA. Primary structure comparisons of this mouse homolog to Drosophila homeotic genes and their encoded proteins demonstrates that it shares homology in both gene structure and in the amino acid sequence of the protein. Messenger RNA analysis shows that the gene is expressed early in embryogenesis and in the central nervous system of both newborn and adult mice.

This information is now serving as a framework for the construction of expression vectors, synthetic peptides, fusion proteins, antibodies and transgenic mice. These tools will be used to determine the functional role of this protein in mouse development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02580-04 LMG
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Determinants of Virus-Host Cell Tropism</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI:                      W. J. Bellini                      Special Expert                      LMG, NINCDS		
COOPERATING UNITS (if any) Neuroimmunology Branch, NINCDS and Dr. Bert Rima, Department of Biochemistry, The Queen's University of Belfast, Belfast, Northern Ireland.		
LAB/BRANCH Laboratory of Molecular Genetics		
SECTION Molecular Virology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">0</div>	PROFESSIONAL: <div style="text-align: center;">0</div>	OTHER: <div style="text-align: center;">0</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <div style="text-align: center; padding-top: 20px;">           This project has been terminated.         </div>		







# ANNUAL REPORT

October 1, 1985 through September 30, 1986

Laboratory of Neural Control, Intramural Research Program  
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT  
October 1, 1985 through September 30, 1986  
Laboratory of Neural Control, Intramural Research Program  
National Institute of Neurological and Communicative Disorders and Stroke

Robert E. Burke, M.D., Chief

Introduction

Research work in the Laboratory of Neural Control (LNLC) is devoted primarily to studies of the central and peripheral neural mechanisms involved in the control of movement in mammals, emphasizing neural organizations at the level of the spinal cord and those regions of the brain stem and cerebral cortex that project directly to the spinal cord.

Present Organization

During FY 1986, the staff of the Laboratory of Neural Control (LNLC) included 14 professional scientists (four permanent senior scientists and ten post-doctoral fellows). The permanent staff also includes three senior support personnel (two engineers and one physiologist), a biological technician, and one laboratory secretary. Non-permanent staff includes three graduate students, one computer programmer, one engineering aide, one laboratory aide, and one Junior Fellow. Because of the close interaction and collaboration among the Laboratory staff, LNLC has not been divided into formal Sections. However, because of the evolution of its program, a request was made in FY 1986 to form two sections: 1) a Section on Neural Mechanisms; and 2) a Section on Neurokinesiology. The research effort can be described under four general headings, divided roughly by methodological approach:

1. Electrophysiological and morphological analysis of the cellular physiology and neuronal circuitry operating in the control of movement at the spinal cord level, largely using acute, reduced preparations (primarily cats).
2. Projects that utilize novel methods for recording the activity of individual neural elements, activity patterns in whole muscles, and kinesiological data in awake, intact animals (both cat and monkey) that are comfortable and performing normal motor behaviors.
3. Theoretical and computer modeling studies of: a.) the electrophysiological properties of identified central nervous system neurons; b.) information processing in neural networks; c.) the mechanical arrangements of bones, joints, and muscles in the cat hindlimb with a view to providing a comprehensive description of their dynamic actions; and d.) the properties of complex proprioceptive elements such as muscle spindles.
4. Activities concerned directly with the development of new instruments and techniques, and the further refinement of existing methods, for recording and analyzing neurally-relevant data from intact, freely moving animals and

for computer-assisted reconstruction of the anatomy of functionally identified neural elements.

### Project Summaries:

Many of the projects underway in LNLIC are interactive with one another, with staff members participating in sub-projects that come under separate headings. Points of overlap between projects included under separate headings will be apparent.

Motor Control Systems in the Spinal Cord: This project and the following one utilize cats, either anesthetized or after decerebration, with destruction of the supratentorial brain. When survival surgery is required, as in studies of the morphology of motor nuclei using retrograde transport methods, surgery is performed under anesthesia and aseptic conditions, and appropriate postoperative care is supplied.

The main direction of this project during FY 1986 was toward analysis of the organization of excitatory interneuronal pathways to motoneurons in the cat spinal cord, with emphasis on the input pathways from distal areas of hindlimb skin. Earlier data suggested that the pathway from cutaneous afferents with low electrical thresholds (less than  $2xT$ ) produce early excitation in flexor digitorum longus (FDL) motoneurons with central latencies consistent with disynaptic connection (less than 1.8 msec). We have now shown that such short latency excitatory components can also be found in certain other hindlimb motor nuclei, although less often than in FDL. The initial excitatory components in cutaneous PSPs are strongly facilitated by stimulation of rubrospinal and pyramidal tract fibers.

We are now using the spatial facilitation method of Lundberg to examine the possible convergence of excitatory drive from the segmental central pattern generator for locomotion onto interneurons in short latency cutaneous pathways. The experiments are done in unanesthetized, decerebrate cats, paralyzed with gallamine, in which rhythmic motor patterns resembling those during normal locomotion ("fictive" stepping) can be elicited by brain stem stimulation, or by administration of L-DOPA after Nialamide pre-treatment. Results to date show marked facilitation of cutaneous EPSP components in FDL motoneurons during the phase of fictive stepping when FDL is normally active (just after extensor turnoff and before flexor activity becomes prominent). Patterns of facilitation in EPSP and IPSP components produced by skin afferent stimulation are being studied in other motor nuclei as well. These experiments are designed to provide information about the organization of excitatory interneuronal pathways to hindlimb motoneurons, and the possible role of these pathways in locomotion pattern generation. The spatial facilitation results will provide key background information for a proposed search for individual interneurons that can: 1) be identified as belonging to particular skin reflex pathways; and 2) that project to particular groups of alpha motoneurons.

The extensive data base available in LNLIC as to detailed morphology of type-identified alpha-motoneurons and of the spatial distribution and numbers of group Ia synapses on such cells has been described in previous Annual Reports. During FY 1986, we utilized these computer models to simulate the steady-state depolarization that would be produced by high-frequency activation of this synaptic system and to compare estimates of the "effective synaptic current" obtained by current injection into the soma with the actual synaptic currents delivered to spatially-dispersed synapses. The experimentally measured "effective synaptic current" underestimates true current delivery by amounts that vary importantly with the distribution of specific membrane resistivity, which cannot be known with precision. These results, produced in response to a request from a scientist outside NIH, illustrate the utility of SPIICE simulation of complex synaptic interactions in realistic neuronal models for gaining increased insight into the interpretation of experimental results.

Intrinsic Properties of Motor Units: We have continued to utilize our extensive data set on the morphology of dendritic trees in type-identified motoneurons, with emphasis on the emerging differences between the branching patterns in the dendrites of cells that innervate fast versus slow twitch muscle units. There are few established methods to examine the topology of dendritic branching and none to deal with the question of quantitative description of how extensive dendritic branching fills space within the "territory" of the dendrite. These questions are of importance to the ways in which synaptic input systems that occupy the same territory make or avoid contact with particular dendrites, and the ways in which dendrites of synergist and antagonist motoneurons interact in 3-space.

Characterization of the motor unit population in the cat tenuissimus (TEN) muscle has continued in collaboration with an investigator at the Hebrew University, Jerusalem, Israel. During FY 1986, the work at NIH has been devoted mainly to analysis of the histochemical profiles of physiologically-typed and glycogen-depleted fibers in single TEN motor units. In addition, we are studying the 3-dimensional arrangement of fibers in the muscle generally and in individual muscle units. The TEN muscle is highly unusual in its long overall length (up to 20 cm), while individual muscle fibers within it are much shorter (up to about 3 cm). This necessitates serial arrangements of muscle unit fibers in order to transmit active force from origin to insertion, but the exact nature of the force transmission system is still unclear. We are attempting to use serial reconstruction methods, developed for neuronal anatomy, to elucidate this problem, which has considerable importance for our other work on the architecture and kinesiology of muscles with complex internal architecture.

Neuromuscular Coordination of Movement: The work in this project is closely associated with that of "Models of Neurophysiological Systems", and they will be described together. These projects include a variety of studies that utilize novel approaches to study motor performance in intact, behaving cats, and to compare actual motor performance with the predictions made by

several theoretical model systems that embody general control principles. Much of the experimental data is obtained using chronically-implanted transducer systems that have been developed and perfected in LNLG over the past decade. The overall goal is to obtain information from intact, freely moving animals that can be related to the existing data base that has been accumulated in anesthetized, immobilized, or otherwise reduced preparations, as well as in a form that can be utilized to refine model systems that test applicability of particular general control theories. These projects also interface importantly with the two projects already discussed above. Device implantation is done under anesthesia and aseptic technique, with appropriate postoperative care. Animals are acclimatized to the laboratory situation before surgery and are trained to walk or run on the treadmill, or on a special runway containing force plates, using affection and food rewards. There is no apparent discomfort associated with the implanted devices or the connector system after the initial postoperative period.

We have continued a detailed analysis of the cat hindlimb musculature with particular reference to structure-function interrelations in bi- or multi-articular muscles. The functional role(s) played by such muscles can vary depending on limb position and often do not fit with simple flexor-extensor, or agonist-antagonist, classifications. Our understanding of the complexity of coordinated muscle action during limb movement has grown considerably during FY 1986, with the increasing sophistication of the computerized cat hindlimb model being developed jointly by LNLG and the Dept. of Electrical Engineering at the University of Maryland (see Contract Narrative N01-NS-3-2348). The development of the cat hindlimb model illustrates very well the utility of a computer model in neuroscience research, not as an end in itself but rather as a guide for thinking and increasingly focussed experiments. To date, simulations of the multiple segment, articulated limb model have shown the critical importance of passive forces (moving masses and gravity) in generating cyclic movement. Limb muscles frequently act to limit movement and recover kinetic energy (i.e., undergo active lengthening), rather than to generate positive work (i.e., active shortening against a load). In some cases, observed muscle action appears anti-intuitive unless considered in the larger context of whole limb kinetics. CNS control of limb movement obviously deals with limb and body kinetics, and it is necessary to consider this context when we attempt to understand the organization of neural control system within the CNS.

During FY 1986, the data base for the hindlimb model has been enlarged in three main areas:

- 1.) Redesigned multi-contact "patch" EMG electrodes with improved spatial directivity have been used to study electromyographic (EMG) activity patterns in intact, moving cats. The activity in particular regions of selected muscles (tensor fasciae lata and sartorius) is correlated with the predominant histochemical fiber type profile in that same muscle region. The relative degrees of recruitment among different motor unit types can then be inferred during a wide range of normal movement behaviors, from steady posture, through

various speeds of locomotion, to large demand movements like scratching and jumping.

2.) Several approaches have been used to examine the distribution of muscle fibers belonging to an individual motor unit (a muscle unit) in muscles with serial fiber arrangements, such as tenuissimus (TEN) and sartorius (SART). The internal architecture of these muscles consists of serial arrangements of muscle fibers with complex interdigitation. Effective transfer of force produced by individual muscle units is a serious problem in such muscles, requiring some mechanism for serial alignment of unit muscle fibers, or a connective tissue matrix that allows serial addition of forces produced by muscle fibers that are much shorter than the overall length of the muscle belly. The physiological approach to this problem involves use of multiple contact EMG electrodes with precise alignment and spacing, fabricated by printed circuit techniques on a flexible insulator substrate. When applied to the surface of sheet-like muscle like TEN and SART, the EMG signals produced by single muscle units can be used to estimate the longitudinal spacing between groups of serially-arranged unit fibers. A second approach uses reconstruction of the 3-dimensional morphology of muscle fibers, sometimes in conjunction with glycogen-depletion of single muscle units, to determine the anatomy of serial interconnections. This approach is time consuming and can be applied only to a small sample of units, but effectively supplements and validates the above physiological technique.

3.) The well-known length-tension characteristics of whole muscles reflect their internal architecture in relation to the relatively fixed functional length range of individual sarcomeres. We have been exploring sarcomere length changes in serial and parallel fibered muscles through the physiological range of muscle length (with muscles in situ), using laser diffraction techniques to estimate mean sarcomere length. The laser diffraction approach has not previously been applied to mammalian muscle in situ, but the approach has proven feasible in our hands.

The problem of neural control of complex muscles has also been investigated in the highly complex muscles that control head and neck movement. This work is done in collaboration with the Department of Physiology, Queens University, Kingston, Canada. Morphological and histochemical studies are done in Canada and the results are compared with physiological and kinesiological observations obtained in LNLC, largely using chronic implant technology. Muscle synergies, reflex effects, and varying degrees of motor unit synchronization are being studied.

We have also continued our studies of the gating of transmission of afferent information ("reflexes") during normal and fictive locomotion and scratching. A study of presynaptic modulation of afferent input to the spinal cord was completed in FY 1986. Surprisingly, there are sufficient levels of presynaptic depolarization (usually associated with "presynaptic inhibition") to produce dorsal root reflexes in extensor muscle afferents during the flexion phase of stepping in both intact and decerebrate cats during

locomotion. This suggests up-modulation of primary afferent depolarization, and presumed presynaptic inhibition, of group Ia afferents to extensor motor nuclei during the flexion phase of stepping, when the extensors are electrically inactive and when the gain of the stretch reflex loop would be expected to diminish.

Theoretical work during FY 1986 has suggested that the tensor analysis approach to motor control is useful for intuitive understanding of the translation that must occur between the coordinate systems that must be embodied in CNS neural organizations, and the quite different coordinate systems that describe limb and body movements in Cartesian space. Testing this idea has been particularly fruitful with the difficult problem of head and neck control, which has a very large potential range of degrees of freedom. Analyses of the properties of muscle spindle afferent discharge, using data from this and other laboratories, suggests that the gamma motor system control of spindle afferent sensitivity is exactly what is required in order to match the very wide ranges of static and dynamic inputs to the relatively narrow frequency bandpass of group Ia afferents (between 20 and 200 Hz) as suggested.

Cortical Mechanisms of Voluntary Motor Control: Work in this project is designed to increase our understanding of the organization of neuronal systems in regions of the cerebral cortex of primates that project directly to the cerebral cortex that have relatively direct pathways to the spinal cord and brain stem (the sensorimotor cortex and supplementary motor area). The major emphasis is on control of arm, wrist, and finger movements and it is for this reason that primates (rhesus monkeys) are used. The animals are intensively trained to perform specific tasks and then chronic chambers are implanted over the appropriate area of contralateral motor cortex, under anesthesia and strict aseptic technique. During daily recording sessions, which usually extend to many months, the animals are minimally restrained in a primate chair and are rewarded for desired performance with fruit juice.

The discharge patterns from individual neurons during arm and hand movements have revealed important features of the neural organization of the primate motor cortex. The physiological experiments combine recording of discharges from single cells during movement performance and during stimulation of various sensory modalities, with intracortical microstimulation of the same localized region of cortex (ICMS), and EMG and kinesiological records of movement performance. Recent results have emphasized the importance of monitoring the electrical (EMG) activity in multiple forelimb muscles during recording of discharge patterns or intracortical microstimulation (ICMS), in order to reveal the presence of inhibitory effects.

Our observations indicate the existence of "colonies" of cortical neurons that produce patterns of excitation and/or inhibition in groups of muscles, rather than in individual muscles. The organization of cortical inhibition, presumably operating through spinal segmental interneuron systems, is of particular interest, since we have found that zones that produce inhibition of



target muscles often border, or surround, zones that produce pure excitation. Much of this complexity had been missed in other studies of cortical organization because multi-muscle EMG methods were not utilized. Extensive maps of cortical areas associated with excitation or inhibition of particular muscles have now been prepared from systematic mapping experiments in individual animals studied over long periods of time. These show that individual muscles are affected by cells within extensive cortical regions, which often exhibit overlap with regions associated with other muscles or muscle groups. These data, considered in relation to cortical inhibition, suggest that the topography of cortical representation is organized in terms of movement patterns rather than individual muscles. In addition, observations on cortical cell discharge patterns during small mechanical perturbations of the manipulandum during practiced movements suggests that the much-debated "long-loop" reflexes may in fact be present in intact animals, and thus must be taken into account in theories of cortical motor control. Preliminary trials have begun to utilize nuclear magnetic resonance scanning to better localize the cortical areas associated with hand and arm movement control.

The flexor carpi ulnaris (FCU) motor pool is of particular interest in this project because its two distinct heads have very different histochemical muscle fiber compositions. In preparation for detailed studies of recruitment sequences in the two heads, the motor pools of the two heads have been labeled by retrograde transport of horseradish peroxidase. The motoneurons supplying the two heads are coextensive within the same motor cell column in the C8 to T2 spinal segments. Thus, if the two heads are used differentially, the segmental control systems that produce different outputs must be differentially distributed without reference to intra-spinal topography.

Collaborative work in this project has been initiated to assess the possible utility of ICMS techniques as the basis for motor or sensory prostheses to aid neurologically-handicapped patients. LNLN staff members will participate with other NIH scientists from BEIB and the Fundamental Neurosciences Program and a team of neurosurgeons in London, Ontario, Canada, to test the effects of ICMS in selected human patients undergoing craniotomy and cortical resection. The aim of this work is to evaluate the quality of sensations perceived by patients during ICMS at stimulus intensities compatible with minimal tissue reaction in animal studies. This work will also utilize improved metal microelectrodes (activated iridium) developed in LNLN.

Techniques for Making Contact with the Nervous System: This project includes all LNLN activities related to the development, design, and fabrication of instrumentation, specialized mechanical equipment, and transducer devices used to support the research work of LNLN. Many of the techniques and instruments developed in LNLN are new and without commercial counterpart. In such cases, LNLN staff attempt to provide assistance to other scientists at NIH and at other institutions around the world who request information and advice about specific data acquisition and processing problems.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 01686-18 LNLG																					
PERIOD COVERED October 1, 1985 through September 30, 1986																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Motor Control Systems in the Spinal Cord																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: R.E. Burke, M.D.</td> <td style="width: 40%;">Chief</td> <td style="width: 20%;">LNLG NINCDS</td> </tr> <tr> <td>Others: J.W. Fleshman, Ph.D.</td> <td>Staff Fellow</td> <td>LNLG NINCDS</td> </tr> <tr> <td>D.E.R. Meyers, Ph.D.</td> <td>Visiting Fellow</td> <td>LNLG NINCDS</td> </tr> <tr> <td>D. Omeniuk, M.S.</td> <td>Student Scientist</td> <td>(3)</td> </tr> <tr> <td>P. Rudomin, Ph.D.</td> <td>Fogarty Scholar-in-residence</td> <td>(2)</td> </tr> <tr> <td>B.J. Schmidt, M.D.</td> <td>Guest Worker</td> <td>LNLG NINCDS</td> </tr> <tr> <td>M. Tokuriki, D.V.M.</td> <td>Guest Worker</td> <td>(1)</td> </tr> </table>			PI: R.E. Burke, M.D.	Chief	LNLG NINCDS	Others: J.W. Fleshman, Ph.D.	Staff Fellow	LNLG NINCDS	D.E.R. Meyers, Ph.D.	Visiting Fellow	LNLG NINCDS	D. Omeniuk, M.S.	Student Scientist	(3)	P. Rudomin, Ph.D.	Fogarty Scholar-in-residence	(2)	B.J. Schmidt, M.D.	Guest Worker	LNLG NINCDS	M. Tokuriki, D.V.M.	Guest Worker	(1)
PI: R.E. Burke, M.D.	Chief	LNLG NINCDS																					
Others: J.W. Fleshman, Ph.D.	Staff Fellow	LNLG NINCDS																					
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B.J. Schmidt, M.D.	Guest Worker	LNLG NINCDS																					
M. Tokuriki, D.V.M.	Guest Worker	(1)																					
COOPERATING UNITS (if any) (1) Dept. of Physiology, Sch. Vet. Med., Yamaguchi Univ., Japan; (2) Dept. of Physiology, CIEA del IPN, Mexico City, Mexico; (3) Dept. of Physiology, George Washington Univ. Sch. Medicine, Washington, DC																							
LAB/BRANCH Laboratory of Neural Control																							
SECTION																							
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892																							
TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 4.5	OTHER: 0.5																					
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             This project is designed to provide information on the mechanisms operating within <u>reflex</u> systems in the adult cat spinal cord, which include <u>alpha motoneurons</u> as the output link, as well as on the interconnections and interactions between reflex pathways and control systems descending to the spinal cord from supraspinal centers. Particular consideration is also given to interrelations between <u>synaptic organization</u>, intrinsic neuronal properties, and dynamic behavior of the alpha motoneurons, and the motor unit type, as defined by the <u>physiological</u> characteristics of the innervated <u>muscle fibers</u>. A variety of preparations have been used, including anesthetized or decerebrate animals as well as intact, freely moving cats. Electrophysiological and morphological data are obtained.           </p>																							

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 NS 01687-18 LNLC</b>																					
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Techniques for Making Connections with the Nervous and Musculoskeletal Systems</b>																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: M.J. Bak</td> <td style="width: 40%;">Electronics Engineer</td> <td style="width: 30%;">LNLC NINCDS</td> </tr> <tr> <td>Others: R.E. Burke, M.D., Chief</td> <td>E.M. Schmidt, Ph.D., Bio Eng</td> <td>LNLC NINCDS</td> </tr> <tr> <td>J.W. Blaszczyk, Visit Fellow</td> <td>W.J. Yee, Bio Eng</td> <td>LNLC NINCDS</td> </tr> <tr> <td>C.M. Chanaud, Guest Res</td> <td>S.L. Wallace, Student Aid</td> <td>LNLC NINCDS</td> </tr> <tr> <td>G.M. Dold, Eng Tech</td> <td>A.J. Rindos, Guest Res</td> <td>LNLC NINCDS</td> </tr> <tr> <td>G.E. Loeb, M.D., Med Off (Res.)</td> <td></td> <td>LNLC NINCDS</td> </tr> <tr> <td>D.E.R. Meyers, Fogarty Fellow</td> <td></td> <td>LNLC NINCDS</td> </tr> </table>			PI: M.J. Bak	Electronics Engineer	LNLC NINCDS	Others: R.E. Burke, M.D., Chief	E.M. Schmidt, Ph.D., Bio Eng	LNLC NINCDS	J.W. Blaszczyk, Visit Fellow	W.J. Yee, Bio Eng	LNLC NINCDS	C.M. Chanaud, Guest Res	S.L. Wallace, Student Aid	LNLC NINCDS	G.M. Dold, Eng Tech	A.J. Rindos, Guest Res	LNLC NINCDS	G.E. Loeb, M.D., Med Off (Res.)		LNLC NINCDS	D.E.R. Meyers, Fogarty Fellow		LNLC NINCDS
PI: M.J. Bak	Electronics Engineer	LNLC NINCDS																					
Others: R.E. Burke, M.D., Chief	E.M. Schmidt, Ph.D., Bio Eng	LNLC NINCDS																					
J.W. Blaszczyk, Visit Fellow	W.J. Yee, Bio Eng	LNLC NINCDS																					
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G.E. Loeb, M.D., Med Off (Res.)		LNLC NINCDS																					
D.E.R. Meyers, Fogarty Fellow		LNLC NINCDS																					
COOPERATING UNITS (if any)  <b>Fundamental Neurosciences Program, NINCDS (F.T. Hambrecht)</b>																							
LAB/BRANCH <b>Laboratory of Neural Control</b>																							
SECTION																							
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, MD 20892</b>																							
TOTAL MAN-YEARS: <div style="text-align: right;">2.0</div>	PROFESSIONAL: <div style="text-align: right;">.5</div>	OTHER: <div style="text-align: right;">1.5</div>																					
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;">           This Project is intended to develop techniques and instrumentation for the acquisition and processing of neuroelectric signals from the central and peripheral nervous system in acute and chronic neurophysiological preparations. Because of this laboratory's continuing interest in sensorimotor neural activity during unrestrained movements, the project also includes development and fabrication of chronically implantable mechanical transducers, catheters, and connectors. Also included is the development of computer programs of general utility for acquisition and analysis of neuroelectric and mechanical records, as well as of neuroanatomical material.         </p>																							

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z0<sup>1</sup> NS 01688-18 LNLC</b>																		
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>																				
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Cortical Mechanisms of Voluntary Motor Control</b>																				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: E.M. Schmidt, Ph.D.</td> <td style="width: 33%;">Biological Engineer</td> <td style="width: 33%;">LNLC NINCDS</td> </tr> <tr> <td>Others: M.J. Bak</td> <td>Electronics Engineer</td> <td>LNLC NINCDS</td> </tr> <tr> <td>G.M. Dold</td> <td>Engineering Technician</td> <td>LNLC NINCDS</td> </tr> <tr> <td>S.R. Goldstein</td> <td>Mechanical Engineer</td> <td>BEI DRS</td> </tr> <tr> <td>F.T. Hambrecht</td> <td>Health Scientist Admin</td> <td>FNP NINCDS</td> </tr> <tr> <td>J.S. McIntosh</td> <td>Physiologist</td> <td>LNLC NINCDS</td> </tr> </table>			PI: E.M. Schmidt, Ph.D.	Biological Engineer	LNLC NINCDS	Others: M.J. Bak	Electronics Engineer	LNLC NINCDS	G.M. Dold	Engineering Technician	LNLC NINCDS	S.R. Goldstein	Mechanical Engineer	BEI DRS	F.T. Hambrecht	Health Scientist Admin	FNP NINCDS	J.S. McIntosh	Physiologist	LNLC NINCDS
PI: E.M. Schmidt, Ph.D.	Biological Engineer	LNLC NINCDS																		
Others: M.J. Bak	Electronics Engineer	LNLC NINCDS																		
G.M. Dold	Engineering Technician	LNLC NINCDS																		
S.R. Goldstein	Mechanical Engineer	BEI DRS																		
F.T. Hambrecht	Health Scientist Admin	FNP NINCDS																		
J.S. McIntosh	Physiologist	LNLC NINCDS																		
COOPERATING UNITS (if any) Fundamental Neurosciences Program, NINCDS (F.T. Hambrecht); Neuroprosthesis Research Program, NINCDS; Univ. Western Ontario, London, Ontario, Canada (Dr. J. Girvin)																				
LAB/BRANCH Laboratory of Neural Control																				
SECTION																				
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892																				
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: .9	OTHER: 1.6																		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;">           This project is designed to investigate the spatial distribution and functional properties of cortical neuron "colonies" in the primate motor cortex that project to the spinal cord and are associated with individual muscles or closely related groups of muscles, as well as the activity of neurons in such colonies during defined voluntary motor behaviors. Intracortical microstimulation (ICMS) is used to map regions that produce excitation or inhibition of particular muscles or muscle groups, and the resultant cortical maps are compared with those for synergist or antagonist muscle groups. To obtain an accurate map, ICMS is done while the animal is performing a task and EMG activity is monitored. <u>Cortical cell discharge patterns</u> during normal movements are evaluated with respect to the excitation or inhibition of muscle activity that is produced by ICMS, and also in terms of EMG patterns. Spinal cord location of motoneurons innervating selected forelimb muscles are studied using retrograde tracing methods. Histochemical studies of muscle fiber types are also performed.         </p>																				

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02079-13 LNLc

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Models of Neurophysiological Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W.B. Marks, Ph.D.	Research Physiologist	LNLc NINcDS
Others:	G.E. Loeb, M.D.	Medical Officer (Res.)	LNLc NINcDS
	M.M. Manley	Bio. Lab. Tech.	LNLc NINcDS
	M.C. Carter, Ph.D.	Staff Fellow	LNLc NINcDS

COOPERATING UNITS (if any)

Dept. of Electrical Engineering, U. MD (W.S. Levine, A.J. Rindos, Jiping He, W.M. Roberts)

LAB/BRANCH

Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION

NINcDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.8

PROFESSIONAL:

1.9

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As quantitative data from a wide variety of techniques and levels of investigation become available for a particular nervous system function, it is both possible and advisable to attempt to assimilate such information into a comprehensive model of the underlying mechanisms and their interactions. This project consists of the development of such models and the necessary analytical and mathematical techniques for their implementation and testing in several areas of intensive experimental investigation by LNLc members and the scientific community at large.

The kinematic model of the cat hindlimb has predicted joint torques which suggest the function of some of the muscles of the hindlimb during locomotion and has revealed patterns of synergies which suggest the existence of underlying functional groupings of muscles. It appears that the tensor notation for parallel processing of sensory and motor signals may be an appropriate language for modelling patterned muscle control systems. Using information theory, we have begun to estimate the optimum relationships of sensitivity to input level for various sensory transducers.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02080-13 LNLC
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neuromuscular Coordination of Movement		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: G.E. Loeb, M.D. Medical Officer, Res LNLC NINCDS Others: W.B. Marks, Ph.D. Research Physiologist LNLC NINCDS J.B. Blaszczyk, Ph.D. Visiting Fellow LNLC NINCDS C.A. Chanaud Guest Researcher LNLC NINCDS S.H. Duenas-Jimenez, M.D. Visiting Fellow LNLC NINCDS C.A. Pratt, Ph.D. Staff Fellow LNLC NINCDS A.J. Rindos Guest Researcher LNLC NINCDS		
COOPERATING UNITS (if any) Queen's University, Dept. of Physiology, Kingston, Ontario, Canada (F.J. Richmond)		
LAB/BRANCH Laboratory of Neural Control		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:  4.6	PROFESSIONAL:  3.4	OTHER:  1.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The cat has long been a standard animal for anatomical and acute physiological studies of muscle function and motor control at the <u>spinal cord level</u>. In this project, a wide variety of traditional and novel <u>kinesiological techniques</u> are being used to study motor tasks in unanesthetized, normally behaving cats, including computer-aided reconstruction of skeletal movement from videotape, multiaxis force plates, chronically implanted nerve cuff and EMG electrodes, and strain and length transducers. The major focus has been the study of <u>hindlimb muscles</u> and their afferent and efferent control during walking, which is the subject of a computer modeling project described in (Project No. Z01-NS-02079-13). Other hindlimb movements studied include jumping, paw shaking, scratching, and reflexes to cutaneous nerve stimulation during normal and decerebrate walking. In a collaborative study, similar data are being collected from a large number of <u>neck muscles</u>.</p> <p>The major objective is to correlate patterns of usage with complex <u>mechanics</u> and compartmentalization and proprioceptive specializations of these muscles. A major theme emerging from these experiments is a concept of "<u>Task Groups</u>," which denotes the segregation and specialization of sensorimotor systems to perform kinematically homogeneous tasks in an optimal manner. This is particularly apparent in <u>multiarticular muscles</u>, which in some cases use independent subdivisions of their alpha motoneuron pool to accomplish kinematically diverse tasks.</p> <p>Recent work has concentrated on functional architecture of muscle units, including mechanical arrangements of their constituent fibers, segregation on the basis of histochemical fiber types, and compartmentalized patterns of EMG recruitment during normal motor behaviors. We believe an overview of these various organizational features of many different motor pools will provide insights into both functional significance of these features and their implications for the spinal segmental circuitry that regulates and coordinates the various muscles.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02160-12 LNLC

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intrinsic Properties of Motor Units

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.E. Burke, M.D.	Chief, LNLC	LNLC NINCDS
Others:	J.W. Fleshman, Ph.D.	Staff Fellow	LNLC NINCDS
	M. Kalia, M.D.	Guest Worker	(2)
	A. Lev-Tov	Visiting Associate	(1)
	D.E.R. Meyers, Ph.D.	Visiting Fellow	LNLC NINCDS
	G.A. Pratt, Ph.D.	Staff Fellow	LNLC NINCDS
	B.J. Schmidt, M.D.	Guest Worker	LNLC NINCDS

COOPERATING UNITS (if any)

(1) Dept. of Anatomy, Hadassah Medical School, Jerusalem, Israel; (2) Dept. of Pharmacology, Jefferson Medical College, Philadelphia, PA

LAB/BRANCH

Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

1.0

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

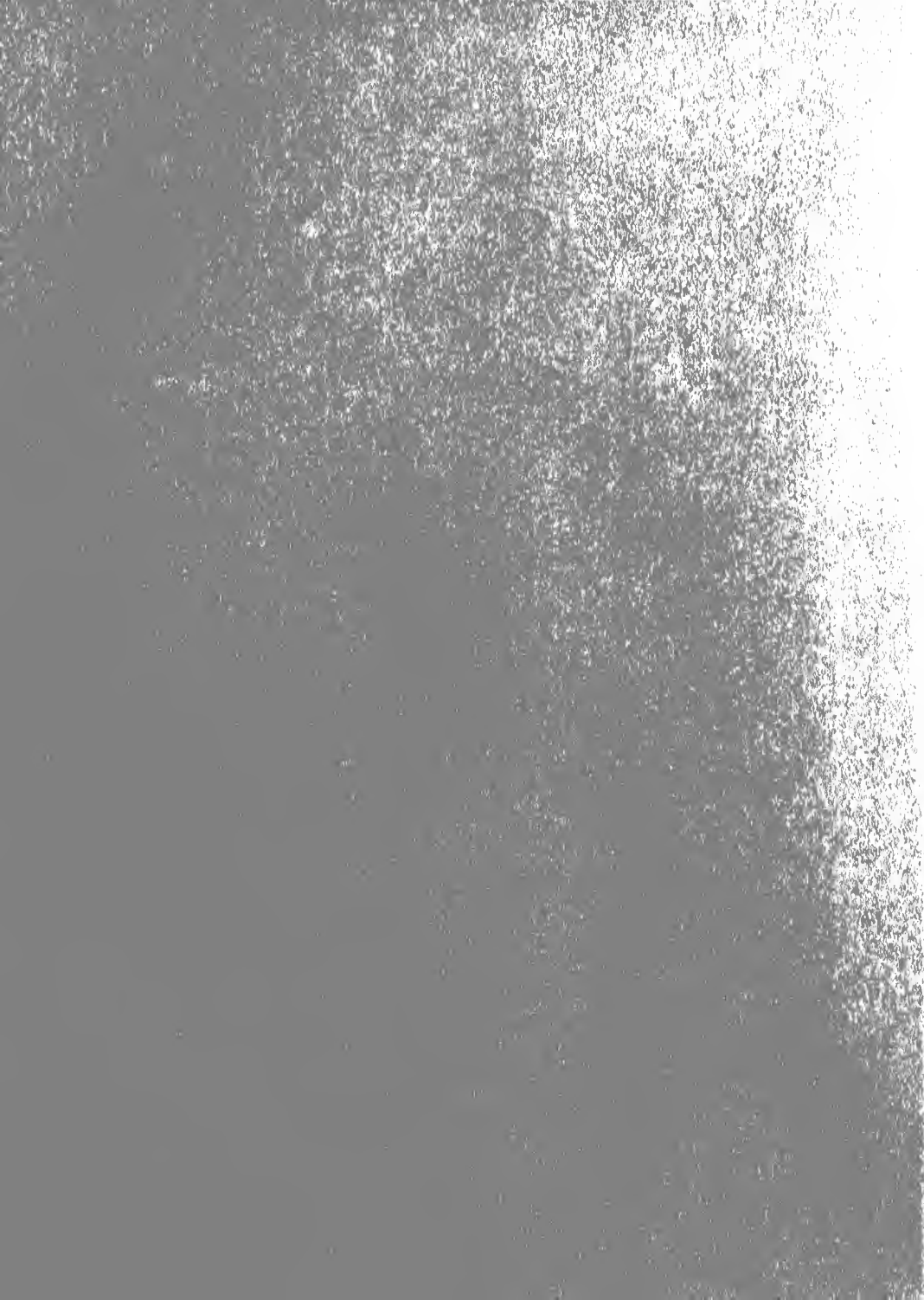
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project is designed to provide information on the ranges and distributions of the electrophysiological and morphological characteristics of alpha motoneurons and of the interrelated mechanical, histochemical and morphological properties of the muscle fibers innervated by them (i.e., the muscle unit) in various hindlimb muscles in the cat. Methods used include intracellular recording and stimulation, measurement of mechanical properties of muscles and individual muscle units, neuroanatomical techniques of intracellular staining with horseradish peroxidase, along with conventional and computer-aided methods for reconstruction of extensive neuronal structures from serial histological sections, and computer modeling and data processing. In some experiments, motor unit populations in normal animals are compared with those in animals after various conditioning treatments. Studies of alpha motoneuron properties are included in this project when they are related importantly to the type of muscle unit innervated by the studied cells.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02534-04 LNL									
PERIOD COVERED October 1, 1985 through September 30, 1986											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Conduction Properties of Peripheral Nerve</b>											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: G.E. Loeb, M.D.</td> <td style="width: 33%;">Medical Officer (Res)</td> <td style="width: 33%;">LNL NINCDS</td> </tr> <tr> <td>Others: C. Krarup</td> <td>Guest Researcher</td> <td>NIB NINCDS</td> </tr> <tr> <td>A.J. Rindos</td> <td>Guest Researcher</td> <td>LNL NINCDS</td> </tr> </table>			PI: G.E. Loeb, M.D.	Medical Officer (Res)	LNL NINCDS	Others: C. Krarup	Guest Researcher	NIB NINCDS	A.J. Rindos	Guest Researcher	LNL NINCDS
PI: G.E. Loeb, M.D.	Medical Officer (Res)	LNL NINCDS									
Others: C. Krarup	Guest Researcher	NIB NINCDS									
A.J. Rindos	Guest Researcher	LNL NINCDS									
COOPERATING UNITS (if any) Neuroimmunology Branch, NINCDS (C. Krarup)											
LAB/BRANCH Laboratory of Neural Control											
SECTION											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892											
TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>The electrical conduction properties of normal and damaged cat peripheral nerves were studied longitudinally using chronically implanted, multi-contact nerve cuff electrodes. The effects of chronic nerve constrictions were similar to clinical dying-back neuropathy, including distal shrinkage and slowing in less severe cases and complete degeneration with proximal dilatation in more severe cases. Regeneration following complete crush lesions could be accurately followed at the single unit level by using stimulus-triggered averaging, with the surprising result that the earliest and fastest regenerating fibers appeared to arise from Group II stem axons rather than the larger Group I fibers. Recovery from a crush combined with a constriction showed significant slowing of both the growth and maturation of regenerating fibers, but eventually recovery was complete.</p> <p>Two papers describing these studies have been submitted for publication. The project has been terminated.</p>											







ANNUAL REPORT

October 1, 1985 through September 30, 1986

Laboratory of Neurobiology  
National Institute of Neurological  
and Communicative Disorders and Stroke

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ANNUAL REPORT  
October 1, 1985 through September 30, 1986  
Laboratory of Neurobiology, IRP  
National Institute of Neurological and Communicative  
Disorders and Stroke

Thomas S. Reese, M.D., Chief

The Laboratory of Neurobiology has two Sections, the Section on Structural Cell Biology and the Section on Structural Plasticity. The Section on Structural Cell Biology uses modern structural and biochemical techniques to investigate basic cell biological problems germane to an understanding of the function of nerve cells; the Section on Structural Plasticity applies these and other appropriate approaches directly to problems of both fundamental and clinical importance in the mammalian central nervous system, emphasizing problems related to regeneration and response to injury. Current emphasis of the Section on Structural Cell Biology is on the mechanism of axoplasmic transport, axonal growth, and synaptic function while the Section on Structural Plasticity is investigating factors which promote establishment of blood-brain-barrier function and neural connections in neural tissues implanted in the brain.

The Section on Structural Cell Biology has recently made progress in understanding the directed organelle movements which move materials by fast axoplasmic transport. Filaments can be isolated from the axoplasm of the squid giant axon which support directed movements of organelles for many hours, at 1-2  $\mu$ m per sec, provided ATP is present. These organelles and filaments are below the resolution of the light microscope so fast digital image processing of differential interference contrast images is required to visualize them; we have had to make several (published) technical improvements in this technique to achieve the results described below. Subsequent direct freezing and metal replication of the filaments showed that they are single microtubules, and that the various organelles moving along them are closely attached.

Treatment of latex beads with a crude extract from squid brain or axon induces the beads, in the presence of ATP, to move along microtubules reconstituted from pure tubulin, suggesting that the neuron contains a free pool of translocator which binds to an organelle and exerts directed forces on microtubules. Affinity for microtubules in AMP-PNP (a nonhydrolysable analogue of ATP) was used to purify from squid brain a 600 KD protein (estimated) with 110 KD and 60-65 KD doublet peptides (4:2 stoichiometry) which, in the presence of ATP, attach to a substrate and induce it to move in one direction along a microtubule. A monoclonal antibody column (directed towards the 110 KD subunit) was subsequently used to purify this translocator from squid brain. Active translocator which eluted off the antibody column at high pH has the same component subunits in the same stoichiometry as the translocator purified by microtubule affinity in AMP-PNP. Thus, the active form of this protein appears to be a polypeptide complex. Molecular shadowing of this complex showed that it is highly asymmetrical, with a large head and a small head connected by a rod resembling a light chain. Which head stably attaches to latex beads, a glass substrate, or an organelle is under investigation.

Based on the results of molecular shadowing as well as results of applying various inhibitors of motile systems, we concluded that the translocator protein is neither a dynein nor a myosin but, instead, represents a new class of motility proteins which we call kinesin. Kinesin also occurs outside of neural cells and may be of general significance in cellular motility. However, organelle movement induced by kinesin has only one direction with respect to microtubule polarity. This direction should be away from the cell body (anterograde), as determined by observing kinesin-induced movement of latex beads along microtubules made from centrosomes, which like the microtubules in the neuron, all have the same defined polarity. Therefore kinesin could only mediate anterograde axonal transport. Indeed, the flow-through from the antibody column, which has been stripped of its kinesin, induces bead movement in a direction that would correspond to retrograde axonal transport. Current efforts are directed at purifying this retrograde translocator, and to understanding how kinesin produces movement.

The movements of microtubules on kinesin-coated glass was analyzed with video microscopy in order to determine how kinesin generates these microtubule-based movements. These movements turned out to depend on the concentration of various nucleotides, metal ions, and of kinesin itself. This approach enables precise manipulation of the chemical motor powering movement using movement itself as the endpoint. The results to date indicate that kinesin-based motility has many properties consistent with it being an ATPase, while other aspects of the mechanochemistry differ from the known properties of myosin and dynein, the other two proteins which mediate intracellular motility, myosin and dynein. We have also developed a method for visualizing in the electron microscope regions of kinesin-coated glass where microtubule movements were previously observed in the video microscope. By combining these biophysical and structural approaches, we hope to establish how kinesin transduces its chemical energy into movement.

In order to develop further a realistic picture of the detailed organization of cytoplasm, monolayers of cultured myocytes and neurons were directly frozen and examined in a 200 kV electron microscope to determine the structure of the cytoplasmic "ground substance" lying between the major filamentous elements and to determine how organelles move through these filamentous elements. This approach has also provided a more detailed understanding of the organization of cytoplasm. A matrix of fine (ca 4 nm) filaments links the major filamentous elements; the soluble proteins and other granular components of cytoplasm are embedded in this fine filament meshwork. Their density and architecture differs in different regions of the cell, and are related to the characteristics of organelle movements in these different regions. Organelles observed moving along microtubule tracks make cross-bridges with microtubules and assume a streamlined, tear-drop shape suggesting that the moving organelle is subjected to viscous forces when it is pulled through the cytoplasmic matrix.

Axon terminals on lizard intercostal muscles are unique in lying close enough to the surface of the muscle to be rapid-frozen, freeze-substituted, and stained with block stains permitting a three-dimensional reconstruction of their cytoplasmic structure. These new freeze-substitution techniques have shown that neurofilament bundles in the axon are continuous, but in the axon terminal they are interrupted by discrete structures (discontinuity plaques) which contain various membrane-limited organelles. These plaques, which are

found throughout the terminal, appear to be specializations for neurofilament degradation and, presumable, are where the filaments transported down the axon are degraded by Ca-activated proteases. The relationship between the distribution of discontinuity plaques and the shape of the terminal indicates that neurofilament degradation may regulate the shapes of functionally distinct types of axon terminals. How proteases, synaptic activity, and extracellular calcium affect the turnover of neurofilaments in the presynaptic terminal is now under investigation.

Direct freezing and improved freeze-substitution techniques have also been applied to growing tips of neuronal processes during development of synaptic connections in the chick optic tectum. Numerous flattened vesicles are found in groups near the growth cone surface; their total area approaches that of the plasmalemma. These membranes would be available to support the rapid expansion of the growth cone surface. We have now shown that these membranes can join the surface membrane at the tip of the growth cone, and that material internalized from the surface ends up in the vicinity of these vesicles. A system for recycling membrane through the plasmalemma of the elongating axonal tip is thus defined by these studies; we have developed a detailed model of the pathways of recycling of membrane through the growth cone.

The serial reconstruction of growth cones also provided reconstructions of individual microtubules. Organelles contacting microtubules were, judging from the results with the squid axon, in transport. The distribution of organelles was uniform along microtubules in axons and proximal growth cones. However, in the mid-segment of the growth cones, the frequency of the small and then the large organelles abruptly dropped, even though the microtubules extended to the ends of the growth cone. Thus, there appear to be discrete loading and unloading zones for axonal transport along the shafts of microtubules in the growth cone.

A set of projects depending on more specialized cryotechniques -- direct freezing, cryosectioning, electron microscopic immunocytochemistry, quantitative x-ray microanalysis, and elemental x-ray imaging -- have yielded new information on the distribution of diffusible elements, including calcium, in cerebellar synapses, and on the role of structural and transport proteins in axons and glia. The x-ray imaging had previously shown that high-calcium sequestration sites were present in highly stimulated preparations only in some of the Purkinje cell dendritic spines. A thorough, quantitative elemental analysis of stimulated and resting cerebellar synapses has now characterized Ca-sequestering organelles operating at normal, regulatory calcium levels. Parallel fiber synaptic vesicles contain less than 0.7 mmol/kg wet weight total calcium in both resting and stimulated preparations, but elevated internal calcium (five-to-sixfold in stimulated synapses and reflecting typical sequestration activity) was found in presynaptic smooth endoplasmic reticulum and in the smooth-membrane cisterns of dendritic spines. These results indicate a role for neuronal endoplasmic reticulum as a calcium-regulatory organelle and, further, imply the involvement of calcium in the formation and maintenance of specialized synaptic contacts. The development of cryosectioning techniques for x-ray microanalysis led to its application in electron microscopical immunocytochemical methods for localizing cytoskeletal proteins in frozen sections of nervous tissues; this new approach was initially applied to a study of myelin structure and

development and then to interactions of axons and glia. The results have implicated several structural and transport proteins, e.g., actin, spectrin and kinesin, in the synthesis, intracellular transport, and insertion of the myelin-specific proteins  $P_0$  and myelin-associated glycoprotein (MAG). Further, a new cytochemical approach, applying in situ hybridization methods to cryosections, has been developed and used to demonstrate differences in the distributions of the m-RNAs encoding for the central nervous system myelin proteins myelin basic protein and proteolipid protein.

The Section on Structural Plasticity has been exploring two aspects of the blood-brain barrier: the role of astroglia in the development of endothelial tight junctions and the routes, as visualized after rapid freezing, by which solutes traverse this endothelium when the barrier is perturbed. The completeness with which astroglia ensheaths the endothelium of cerebral capillaries suggests that there might be a mutual interaction that could modulate the structure of endothelial plasma membranes. In order to test this notion, we have maintained these two cell types in vitro, separately and together. It is known that cerebral endothelial cells (bovine) in primary culture are able to form tight junctions, some of which exclude ionic lanthanum. However, such primary cultures are contaminated by astroglia, oligodendroglia and other cells. We have found that in highly enriched subcultures of beef brain endothelium, about 90% of the cells can be identified as endothelial. Their junctions, viewed in freeze-fracture replicas, differed from those in situ in two ways: the tight junctions were reduced from continuous, interconnected strands to a few, fragmentary strands; numerous gap junctions, known to be present in immature cerebral capillaries but never in mature capillaries, lay adjacent to the strands. When the endothelial cells were co-cultured with secondary, enriched (70-90%) astroglia derived from two day old rats, both tight and gap junctions were affected. The tight junctions of some endothelial cells were greatly enhanced: their strands were longer, they formed many more anastomotic rows, and they were continuous, a prerequisite for a barrier type of junction. The distributions of both the lengths and widths of tight junctions in solo endothelial cultures as compared to co-cultures were significantly different ( $p < 0.005$ ). The second effect was a consistent reduction in the area occupied by gap junctions. Thus, by enhancing tight junctions and diminishing gap junctions, the astroglia was able to "normalize" the endothelial junctions in vitro and, presumably, could do so in vivo. That the effects are specifically astroglial is suggested by the failure of fibroblasts or aortic smooth muscle cells, when substituted for astroglia, to affect the endothelial junctions. More appropriate cells, which remain to be substituted for astroglia, are pericytes and pial smooth muscle cells. Because the astroglial influence was exerted on the cerebral endothelium of another species, it may reflect a fundamental property of a class of astrocytes. However, the astroglia had no effect on the tight junctions of endothelium from other organs, even in the same species. To see whether other cells in turn, affect astroglial functions, we will determine whether the level of glutamine synthetase activity is elevated when astroglia is co-cultured with appropriate neurons as compared to endothelium.

We have further tested the possibility that the particle assemblies in the astrocytic plasma membrane are tethered to the cytoskeleton by chemically disrupting this component of astrocytes and then subjecting them to electrophoresis. The assemblies were not redistributed after such drastic



treatment and may, therefore, either have no association with mobile components of the cytoskeleton, do not have a net electric charge, or are unable to migrate in the lipid bilayer of the cell membrane.

A second project, also related to the blood-brain barrier, focuses on structural alterations in perturbed cerebral endothelium preserved by cryofixation. Since one of the most interesting perturbations is cell shrinkage, the technique of freeze-substitution, which minimizes movement of tissue water and solutes, is especially appropriate. The pial vessels of frogs are one of a few examples of capillaries accessible to direct freezing. Four groups of animals were examined: (1) normothermic; (2) hypothermic; (3) hyperosmotic (3M urea was applied to the pia of normo- and hypothermic frogs); and (4) injured by a cold probe. Thin plastic sections of the freeze substituted brains were cut for morphometry and serial reconstruction.

The endothelium of most capillaries in all groups was deeply indented by large luminal and abluminal pits. Although some of these pits fused with cytoplasmic "vesicles", none that were traced in serial sections turned out to be transcellular channels. In the hyperosmotic groups, some of the clefts between endothelial cells were patent and allowed ferritin to pass from blood to basement membrane. The pits and open clefts were not formed by autolytic changes that had taken place in the excised pieces of brain, because these configurations were also present in hypothermic tissue. The route by which solute can passively move across shrunken endothelium is, therefore, pericellular rather than transcellular. Although mammalian pial vessels have a tunica media, this layer may be thin enough not to interfere with optimal freezing of the subjacent endothelium. If so, cryofixation may offer a most promising means of revealing pathological changes in mammalian blood vessels that may not be apparent after conventional, aldehyde fixation.

A third major endeavor is to delineate, in situ, the role of extracellular matrix and target tissue in the regeneration of central, mature axons. The laminin component of extracellular matrix promotes the elongation of neurites in vitro. We have recently demonstrated that cerebral tissue can grow a short distance into an empty metal tube while elaborating its own extracellular matrix. Can axonal regeneration be accelerated and enhanced if the conduits are prefilled with either purified laminin, crude extract of Schwann cell extracellular matrix, or culture medium? Polyethylene tubes, bent into U shapes, were fitted with a side tube attached to an indwelling osmotic pump which delivered Schwann cell matrix for one to two weeks. One end of the tube was inserted into the corpus callosum of adult rats, the other end, containing 16 day-old fetal association cortex as target tissue, was inserted into a lateral ventricle where the sprouting vessels of damaged choroid plexus might be a source for graft revascularization.

To date, none of the media enhanced axonal regeneration beyond that which takes place in an empty, metal tube. However, even after five months, some regeneration continued, as manifested by an occasional growth cone. Although many astrocytic processes were oriented longitudinally and could serve as a guidance scaffold for elongating axons, some growth cones contacted axons rather than astroglia.

While capillaries readily grew into the grafts in the different media, and could provide nutrients to ingrowing tissue, the use of porous tubes, such

as silastic, collagen and polylactate, might enhance this process by permitting gas exchange before the arrival of new vessels. Another approach we plan to take is to introduce astrocytes, Schwann cells or endothelial cells, in addition to fetal target cells, into the tubes to compare their effects on axonal regrowth. We have begun work designed to determine whether there is neurovascular specificity. Tissues with different types of capillaries are inserted into the hypothalamohypophyseal tract in order to compare the pattern and degree of neurovascular association.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 NS 01442-20 LN

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Permeability of Cellular Layers in the Vertebrate Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: T.S. Reese, M.D., Chief LN, NINCDS  
Others: S.B. Andrews, Ph.D. Special Expert LN, NINCDS  
J. Frokjaer-Jensen, Ph.D. Visiting Associate LN, NINCDS  
B. Kachar, M.D. Visiting Associate LN, NINCDS

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543

J.S. Handler, KE, IR, NHLBI, NIH, Bethesda, MD

R.P. Rand, Brock University, St. Catherine's, Ontario, Canada

R.C. Wagner, University of Delaware, Newark, DE

LAB/BRANCH

Laboratory of Neurobiology

SECTION

Section on Structural Cell Biology

The Marine Biological Laboratory, Woods Hole, MA 02543

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.9

PROFESSIONAL 0.8

OTHER

1.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The substructure of tight and gap junctions is investigated by direct freezing techniques that avoid any chemical fixation and serve to increase the definition of individual membrane components. A new model of tight junction structure was developed which replaced the previous view these junctions are comprised of rows of intramembrane proteins; rod-shaped structures seen after direct freezing are now interpreted as inverted cylindrical micelles of membrane lipids. Evidence for this model has been gathered from investigations of pure lipid bilayer systems which are induced to form non-planar micellar phases by addition of calcium ion. Cylindrical lipid micelles identical to the cylinders at tight junctions are found embedded in these lipid bilayers. Tight junctions, but not septate junctions, in invertebrates also appear to have lipidic backbones. How tight junctions prevent small charged solutes from entering the brain (across the blood-brain barrier) is made clear by this new model of tight junction structure. Gap junctions form within minutes of treating cells with certain mild lipid solvents, suggesting that precursors of the intramembraneous components of gap junctions are continuously present in the cell membrane. Direct freezing techniques were also used to investigate the distribution of endothelial vesicles and other intracellular organelles in capillaries that had not been subjected to chemical treatment or fixation. Approximately threefold fewer apparently free vesicles were found in directly frozen endothelial cells, as compared with aldehyde-fixed cells, from capillaries of the eel swim bladder and also from cultured human endothelium. However, ultrathin serial sectioning showed that in both cryofixed and chemically fixed eel capillaries, as in the capillaries of the frog mesentery, virtually all vesicles are in continuity with the plasma membrane.

7 - LN/NINCDS

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 01881-16 LN												
PERIOD COVERED <div style="text-align: center;">October 1, 1985 through September 30, 1986</div>														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <div style="text-align: center;">Structural Basis of Synaptic Transmission</div>														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.: T.S. Reese, M.D.</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">LN, NINCDS</td> </tr> <tr> <td>Others: T. Cheng, Ph.D.</td> <td>Visiting Fellow</td> <td>LN, NINCDS</td> </tr> <tr> <td>B. Kachar, M.D.</td> <td>Visiting Associate</td> <td>LN, NINCDS</td> </tr> <tr> <td>J. Walrond, Ph.D.</td> <td>Staff Fellow</td> <td>LN, NINCDS</td> </tr> </table>			P.I.: T.S. Reese, M.D.	Chief	LN, NINCDS	Others: T. Cheng, Ph.D.	Visiting Fellow	LN, NINCDS	B. Kachar, M.D.	Visiting Associate	LN, NINCDS	J. Walrond, Ph.D.	Staff Fellow	LN, NINCDS
P.I.: T.S. Reese, M.D.	Chief	LN, NINCDS												
Others: T. Cheng, Ph.D.	Visiting Fellow	LN, NINCDS												
B. Kachar, M.D.	Visiting Associate	LN, NINCDS												
J. Walrond, Ph.D.	Staff Fellow	LN, NINCDS												
COOPERATING UNITS (if any)  <div style="text-align: center;">           Marine Biological Laboratory, Woods Hole, MA 02543            D. Landis, Dept. of Neurology, Massachusetts General Hospital, Boston, MA            R.A. Altschuler and J. Fex, LNO, NINCDS, NIH, Bethesda, MD         </div>														
LAB BRANCH <div style="text-align: center;">Laboratory of Neurobiology</div>														
SECTION <div style="text-align: center;">Section on Structural Cell Biology</div>														
INSTITUTE AND LOCATION <div style="text-align: center;">The Marine Biological Laboratory, Woods Hole, MA 02543</div>														
TOTAL MAN-YEARS <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; text-align: center;">2.9</td> <td style="width: 33%; text-align: center;">1.9</td> <td style="width: 33%; text-align: center;">1.0</td> </tr> </table>			2.9	1.9	1.0									
2.9	1.9	1.0												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>A method for staining freeze-substituted tissue has been developed which requires no further stain after the sections are cut, so the stain extends evenly through the section. Therefore the three-dimensional structure of the cytoskeleton and related fine filaments in synapses can be determined in continuous serial sections. How neurofilaments end in synaptic terminals has been determined; this is important because neurofilament lengths are thought to be regulated by Ca-activated proteases at their terminations. Growing nerve terminals in the brain have been reconstructed from serial sectioned freeze-substituted preparations. These new preparative methods have revealed an internal system of membranes which are thought to be the source of the new membrane added to the surface of the growth cone during its growth. These membranes are highly labile and are destroyed by conventional fixatives. Current evidence indicates that they participate in recycling of membranes needed for extension of the growth cone as well as function as intracellular compartments where the plasmalemmal surface of the growth cone can be modified during neuronal development. New collaborative work on auditory hair cells with video microscopy shows the distribution there of transmitter enzymes as well as shape changes in living hair cells during transduction. Another collaborative study has used immunofreeze-fracture to show that the distribution of acetylcholine receptors in lipid vesicles corresponds to particles protruding from their membranes; this is preliminary to a study of acetylcholine receptor structure.</p>														
8 - LN/NINCDS														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 NS 02551-05 LN

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Structure of Neuronal Cytoplasm

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.:	T.S. Reese, M.D.	Chief	LN, NINCDS
Others:	T.P.O. Cheng, Ph.D.	Visiting Fellow	LN, NINCDS
	P. Gallant, Ph.D.	Special Expert	LN, NINCDS
	B. Kachar, M.D.	Visiting Associate	LN, NINCDS
	B.J. Schnapp, Ph.D.	Staff Fellow	LN, NINCDS
	M. Terasaki, Ph.D.	Staff Fellow	LN, NINCDS
	R. Vale, Ph.D.	Staff Fellow	LN, NINCDS

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543  
M. Sheetz, Univ. of Connecticut Health Center, Farmington, CT

LAB/BRANCH

Laboratory of Neurobiology

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINCDS, NIH,  
The Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL MAN-YEARS

5.0

PROFESSIONAL

3.0

OTHER

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project determines the structure of neuronal and glial cytoplasm, particularly as it pertains to axoplasmic transport, and the organization of cytoplasm. Cultured myocytes grown on grids, frozen, freeze-substituted, and examined directly at high voltages in an electron microscope have a cytoplasmic ground substance consisting of fine filaments instead of a microtubular meshwork, and distinct cytoplasmic domains characterized by organelle movements along microtubules. Microtubules isolated from the axoplasm of the squid giant axon continue to support movements of organelles for many hours. However, actin filaments in other cells support similar movements of organelles. A protein translocator responsible for these organelle movements has been characterized, a 600 KD protein with 110 KD and 60-65 KD doublet peptides. This protein induces beads to move along purified microtubules in the presence of ATP. When a monoclonal antibody column (directed towards the 110 Kd subunit) is used to purify this translocator it has the same peptide components and supports similar movements. Based on its size, pharmacological properties, and asymmetrical shape seen by molecular shadowing, this translocator protein belongs to a new class of motility protein, which we call kinesin. Kinesin appears to be of general significance in cellular motility. The organelle movements induced by kinesin are always directed towards the plus ends of microtubules, a direction corresponding to anterograde axonal transport. However, brain extracts stripped of kinesin with monoclonal antibody induce bead movement in the retrograde direction. Our current efforts are concentrated on purifying the translocator for retrograde movements and on determining whether organelles selectively bind translocators in order to explain how they are selected for anterograde or retrograde transport.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02610-03 LN												
PERIOD COVERED <p style="text-align: center;">October 1, 1985 through September 30, 1986</p>														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>The Distribution of Mobile and Structural Components at Chemical Synapses</b>														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.</td> <td style="width: 33%;">S.B. Andrews, Ph.D.</td> <td style="width: 33%;">Special Expert</td> <td style="width: 15%;">LN, NINCDS</td> </tr> <tr> <td>Others:</td> <td>T.S. Reese, M.D.</td> <td>Chief</td> <td>LN, NINCDS</td> </tr> <tr> <td></td> <td>K.G. Herman, Ph.D.</td> <td>Staff Fellow</td> <td>LN, NINCDS</td> </tr> </table>			P.I.	S.B. Andrews, Ph.D.	Special Expert	LN, NINCDS	Others:	T.S. Reese, M.D.	Chief	LN, NINCDS		K.G. Herman, Ph.D.	Staff Fellow	LN, NINCDS
P.I.	S.B. Andrews, Ph.D.	Special Expert	LN, NINCDS											
Others:	T.S. Reese, M.D.	Chief	LN, NINCDS											
	K.G. Herman, Ph.D.	Staff Fellow	LN, NINCDS											
COOPERATING UNITS (if any) C.E. Fiori and R.D. Leapman, BEIB, DRS, NIH, Bethesda, MD. D.M.D. Landis, Case-Western Reserve University, Cleveland, OH. B.D. Trapp, Johns Hopkins University School of Medicine, Baltimore, MD.														
LAB BRANCH Laboratory of Neurobiology														
SECTION Section on Structural Cell Biology														
INSTITUTE AND LOCATION The Marine Biological Laboratory, Woods Hole, MA 02543 NINCDS, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">2.4</td> <td style="width: 33%;">1.4</td> <td style="width: 33%;">1.0</td> </tr> <tr> <td style="text-align: center;">PROFESSIONAL</td> <td style="text-align: center;">OTHER</td> <td></td> </tr> </table>			2.4	1.4	1.0	PROFESSIONAL	OTHER							
2.4	1.4	1.0												
PROFESSIONAL	OTHER													
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           This project aims to determine the intracellular distribution of diffusible and structural components within axons, dendrites, glia, and synapses. This work is important because of the relationship between the localization and movement of cellular constituents and their role in synaptic transmission. This project depends on several recent technological advances, including direct freezing, cryosectioning, immunocytochemistry in ultrathin cryosections, and quantitative x-ray microanalysis and element-specific x-ray imaging. Quantitative studies of the intracellular calcium distribution in parallel fiber/Purkinje cell cerebellar synapses indicate that total calcium is below 0.7 mmol/kg wet weight in all resting pre- and postsynaptic organelles; thus, high-level calcium stores appear unnecessary for the activity of these synapses. Membrane depolarization, however, elicits a fivefold increase in (extracellularly derived) calcium in the smooth endoplasmic reticulum of presynaptic terminals and dendritic spines, while that in synaptic vesicles is unchanged, thereby identifying the endoplasmic reticulum as a site of calcium sequestration. Immunocytochemical studies using frozen sections of actively myelinating nerve have implicated cytoskeletal and transport proteins, e.g., actin, spectrin and kinesin, in the synthesis and insertion of the myelin-specific proteins P<sub>0</sub> and myelin-associated glycoprotein. Similarly, a new approach using <u>in situ</u> hybridization methods in frozen sections has revealed the differential localization of m-RNAs encoding for the central nervous system myelin-specific proteins myelin basic protein and proteolipid protein. Thus, this project continues to provide important new information on the detailed relationship between the diffusible and structural components of neurons and glia, and how these regulate neuronal activity.         </p>														

10 - LN/NINCDS

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02700-01 LN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Mechanochemistry of Proteins Involved in Axonal Transport		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.      B.J. Schnapp, Ph.D.      Senior Staff Fellow      LN, NINCDS  Others:   S.H. Kahn, Ph.D.      Guest Researcher Albert Einstein College of Medicine, Bronx, NY T.S. Reese, M.D.      Chief      LN, NINCDS		
COOPERATING UNITS (if any)  M.P. Sheetz, Washington University, St. Louis, Mo.		
LAB/BRANCH Laboratory of Neurobiology		
SECTION Section on Structural Cell Biology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.2	PROFESSIONAL: 1.2	OTHER 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The goal of this new project is to understand how the motors which power fast axonal transport transduce the chemical energy associated with the hydrolysis of ATP into directed movement of organelles along microtubules. We began by analyzing the sliding of microtubules along glass coated with kinesin, the protein which powers anterograde transport. In order to understand how kinesin works in this simple system, we addressed three problems: to define the sequence of chemical reactions which comprise the mechanochemical cycle; to define the different structural configurations which kinesin undergoes during the work cycle; to determine how the chemical cycle is coupled to the work cycle. The heart of this project involves analyzing microtubule based motility by video microscopy, using a digital processor to both generate images with sufficient contrast to visualize single microtubules and to acquire and quantitatively analyze motion data. Motion is analyzed as a function of biochemical manipulations in a flow cell. This information complements conventional biochemical measurements characterizing the binding of kinesin to microtubules and of nucleotide substrates and products to kinesin. Electron microscopy of rapidly frozen kinesin on glass is used to determine structural configurations of transient intermediates. These investigations define fundamental biophysical properties of kinesin-based movement which will serve as a baseline for assaying the effects of other proteins in cytoplasm which interact with and perhaps regulate kinesin. Finally, our ability to combine, in a single experiment, biochemistry with motion analysis and electron microscopy using purified components promises to make a major contribution toward understanding the fundamentals of force transduction in motility systems.		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 01805-18 LN
PERIOD COVERED <p style="text-align: center;">October 1, 1985 through September 30, 1986</p>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <p style="text-align: center;">Astrocyte-Endothelial Interactions * *</p>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
P.I.	J.H. Cheng, Ph.D.	Staff Fellow  LN, NINCDS
Others:	Z. Nagy, M.D. M. Brightman, Ph.D.	Visiting Associate Head, Section on Structural Plasticity  LN, NINCDS LN, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH <p style="text-align: center;">Laboratory of Neurobiology</p>		
SECTION <p style="text-align: center;">Section on Structural Plasticity</p>		
INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, MD 20892</p>		
TOTAL MAN-YEARS <p style="text-align: center;">2.4</p>	PROFESSIONAL <p style="text-align: center;">1.6</p>	OTHER <p style="text-align: center;">0.8</p>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           Because the cerebral endothelium is so completely ensheathed by astrocytes, it is possible that the two cell types interact so as to modulate the structure of their plasma membranes. The capillary endothelial cells have complex, zonular tight junctions (TJ), and the perivascular astrocytic membranes have localized high concentrations of particle assemblies (300-500/<math>\mu\text{m}^2</math>). When the endothelial cells were separately grown as enriched subcultures, the constituent strands of the endothelial TJ, viewed in freeze fracture, were reduced to a few, isolated patches. Among the strands lay many gap junctions which, <u>in situ</u>, are known to be absent from mature, brain capillaries. When the two cell types were co-cultured, the endothelium had greatly enhanced TJ: broad arrays of long, continuous, interconnecting strands. The distributions of both TJ length and width between solo endothelial cultures and co-cultures were significantly different (<math>p &lt; 0.005</math>). The area occupied by gap junctions was markedly reduced from a mean of 27% in solo, endothelial cultures, to 8% in co-cultures. The endothelial junctions were thus "normalized" by the astroglia. Substitution of astroglia with fibroblasts or aortic smooth muscle cells had no effect on the TJ. The astrocytes, in solo culture had randomly distributed, low numbers of assemblies (1/<math>\mu\text{m}^2</math>), which, in co-culture became aggregated while the assembly density of the non-aggregated areas increased 5 fold. The assemblies of astrocytes, grown on purified laminin or with fibroblasts instead of endothelium, were unaltered. Thus, <u>in vitro</u> and, perhaps <u>in vivo</u>, the astroglia may modulate endothelial junctions involved in the blood-brain barrier, while the endothelium affects the number and distribution of assemblies within the astrocyte cell membrane.            * *(Formerly "Membrane Structure of Astrocytes")         </p>		
12- LN/NINCDS		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02086-13 LN

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Regeneration in Transplanted Peripheral and Central Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. N. Azzam, Ph.D.

Guest Researcher

LN, NINCDS

Others: M. Brightman, Ph.D.

Head,

LN, NINCDS

Section on Structural Plasticity

D. Carey, Ph.D.

Guest Researcher

Dept. of Physiology, Hershey Medical Center

COOPERATING UNITS (if any)

Dept. of Physiology, Milton S. Hershey Medical Center

LAB/BRANCH

Laboratory of Neurobiology

SECTION

Section on Structural Plasticity

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.5

PROFESSIONAL

1.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type Do not exceed the space provided)

The laminin component of extracellular matrix is known to promote neuritic extension in vitro. We have previously shown that mature axons of the corpus callosum can regenerate for a limited distance through initially empty metal tubes that eventually became filled with the extracellular fluid provided by the tissue growing into them. We are now asking whether the regeneration of these axons can be enhanced in tubes pre-filled with tissue culture medium, a purified laminin gel, or a crude extract of Schwann cell extracellular matrix. A polyethylene tube (PE-50), shaped in the form of a U, was fitted with a narrow side-arm tube (PE-10), through which the solution of Schwann cell matrix was delivered by an indwelling, osmotic pump over a 1 - 2 week period. Fetal (E-16 days old) association cortex, as target tissue, was placed in one end of the U - shaped tube which was inserted into a lateral ventricle of an adult rat. The opposite end was placed in the contralateral corpus callosum. After 2-20 weeks, the media used had not enhanced axonal regeneration beyond that obtained with empty conduits. Only small fragments of fetal tissue survived within the tubes. However, bundles of remyelinating axons, with thin myelin sheaths and heminodes, and unmyelinated axons entered the tubes for a short distance. Even after 5 months, regeneration continued as indicated by the entry of a few growth cones. Astrocytes entering the tube not only aggregated into gliotic tangles, but were also oriented in the long axis of the tube. While the oriented astocytic processes might serve as a scaffold for elongating axons, some growth cones were in contact with axons and did not appear to use the astroglia for guidance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02144-12 LN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders) The Blood-Brain Barrier		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: M. Brightman, Ph.D.  Others: Z. Nagy, M.D. K. Pettigrew, Ph.D.	Head, Section on Structural Plasticity  Visiting Associate Guest Worker Research Mathematical Statistician	LN, NINCDS  LN, NINCDS NIMH, NINCDS
COOPERATING UNITS (if any)  N.I.M.H.		
LAB BRANCH Theoretical Statistics and Mathematics Branch		
SECTION Section on Structural Plasticity		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 1.5	PROFESSIONAL 1.2	OTHER .3
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>             The routes by which solutes traverse a perturbed cerebral endothelium are, arguably, transcellular or intercellular. Rapid freezing rather than aldehyde fixation, during which cytoplasmic vesicles continue to fuse, may capture the momentary formation of either route. The only cerebral vessels thin enough and accessible for optimal freezing are pial capillaries, present in frogs but not mammals. Accordingly, the pial surface of frogs that were (a) normothermic (22°C), (b) hypothermic (5°C for 3-5 days), (c) normo- or hypothermic and exposed topically to hyperosmotic urea (2.5-3M) and (d) injured by a cold probe (-70°C) applied to the skull were rapidly frozen. About 50 mg ferritin was infused intravenously in groups (a), (b) and (c). Thin plastic sections were examined morphometrically. The endothelium of all groups was indented by large, luminal and abluminal pits 0.08-0.32 <math>\mu\text{m}</math> wide, which communicated with some cytoplasmic vesicles but did not form transcellular channels. The number of pits in the normal frogs (5/<math>\mu\text{m}^2</math> of membrane) was similar to the hyperosmotic group (4/<math>\mu\text{m}^2</math> of membrane) but half of that in the cold lesion groups (10/<math>\mu\text{m}^2</math> of membrane). In the endothelium of the hyperosmotic groups, both normothermic and hypothermic, the intercellular clefts were patent instead of being sealed by tight junctions. Ferritin traversed such clefts to enter the perivascular basement membrane. The pits and open clefts were not autolytic features of the excised pieces of brain because they also typified hypothermic tissue. The route of passive permeation across cerebral endothelium during hyperosmotic treatment is, therefore, paracellular rather than transcellular.           </p>		





ANNUAL REPORT

October 1, 1985 through September 30, 1986

Laboratory of Neurochemistry  
National Institute of Neurological and  
Communicative Disorders and Stroke

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ANNUAL REPORT  
October 1, 1985 through September 30, 1986  
Laboratory of Neurochemistry, Intramural Research  
National Institute of Neurological and Communicative  
Disorders and Stroke  
R. Wayne Albers, Ph.D., Acting Chief

The Laboratory of Neurochemistry is composed of two Sections: Enzyme Chemistry, and Neuronal Development and Regeneration.

Studies of the mechanisms and regulation of cation transport constitute the major project in the Section on Enzyme Chemistry. Other projects in the section relate to secretory mechanisms, and to nerve regeneration.

A number of new approaches to long-standing interests of this Section with respect to the structural basis of ion transport have become practicable within the past year because data on the complete amino acid sequences of the Na,K-ATPase and of several related transport ATPases have become available. A series of antibodies directed against known ATPase sequences are being developed as structural and functional probes of the Na,K-ATPase. These antibodies are being employed in the characterization of the transport system. Current studies are directed toward the identification of domains of the ATPase that make up the cation binding and transport sites. Evidence suggesting the widespread occurrence of multiple forms of the sodium pump is also being investigated.

The secretion project of the Section on Enzyme Chemistry involves studies of the process of granule activation and secretion in mast cells. Of particular interest is the high calcium content within mast cell secretory granules. Evidence has been obtained which suggests that the matrix of these granules contains preformed membrane components that may be rapidly inserted into the granule membrane during the activation process. Current work has provided evidence for the transfer of phospholipid from the granule matrix to the granule membrane. These studies suggest that similar processes may be important for the control of secretion as it relates to neurotransmitter release.

A collaborative project on quantitative studies of nerve regeneration has been initiated. Nerve regeneration can be logically considered in four aspects: 1) the peripheral signal; 2) the neuronal growth response; 3) axonal guidance; and 4) re-establishment of synapses. Current work is directed at quantitatively evaluating the neuronal growth response in peripheral nerves as reflected by the changes in neurofilament proteins. Baseline data have been acquired and experiments in progress suggest that the further analysis of neurofilament protein composition in different stages of nerve response to injury can provide a useful index of the regenerative process.

The Section on Neuronal Development and Regeneration has devoted all of its efforts to investigating the factors that regulate the use of foreign nervous tissue grafts to aid in the repair of injured nervous tissue. Experiments were performed to (1) evaluate the effectiveness of the immunosuppressive agent Cyclosporin-A (Cy-A) in preventing the rejection of cross-species (i.e., xenografts) neuronal grafts, (2) determine what is the prime target tissue of the immune response directed towards a nerve allograft, (3) investigate the status of the blood-nerve barriers in nerve allografts during Cy-A immunosuppression, and (4) explore the feasibility of using cryopreserved rather than frozen, killed nerve grafts to repair injured nerve. The Section has also begun to analyze many aspects of these studies with grafts by electron microscopy, and since this is a new technique in the Section, considerable time has been spent gathering morphological data on normal and transplanted nervous tissue. Because of the importance of the experiments with grafts, no time was available to continue work on trophic interactions of neurons and target tissues. Accordingly, the project on trophic interactions will be terminated.

The data from the aforementioned studies with grafts demonstrated that Cy-A prevented the rejection of hamster and mouse but not guinea pig neurons in rats. However, neurons were rejected during Cy-A treatment in all other donor-host combinations (e.g., hamster to mouse). The effect of Cy-A on neuronal xenografts is unpredictable, and clinical implications drawn from a single animal donor-host combination must be guarded. Since it is likely that xenoantibody causes the rejection of most cross-species grafts, plans are in progress to determine whether the antibody is preformed or arises after grafting. Once this data is obtained methods may be found to eliminate it. Other results indicated that the vascular system of a nerve allograft was the first tissue reacted upon by the immune response. The vessels of the allograft were found to be necrotic and thrombosed. Thus, immune infarction appears to be the reason why donor Schwann cells disappear from a nerve allograft thereby making it a poor pathway for regenerating host axons. The status of the endoneurial blood-nerve and perineurial-nerve barriers of nerve allografts during Cy-A immunosuppression was examined with the tracer horseradish peroxidase (HRP). After intravenous injection, this enzyme can be localized by light and electron microscopy to determine any changes in permeability to it by the two barriers of nerve. The endoneurial graft vessels became permeable to HRP during the period of Wallerian degeneration in the graft, but they restored their normal impermeability to it after host axons that had regenerated through the graft became remyelinated. Interestingly, the perineurial barrier to HRP remained intact throughout the period of graft survival. Accordingly, no nerve barrier abnormalities persist in surviving nerve allografts which prejudice their usefulness. The establishment of a nerve bank from which to draw unlimited quantities of nerve is a desirable goal. Since frozen, killed nerves function poorly as conduits for host axonal growth, a study was undertaken to determine the feasibility of cryopreserving grafts (i.e., freezing them in an agent that prevents ice



damage to cells so that they remain viable after thawing). It was found that the cryoprotectant dimethyl sulfoxide permitted cryopreservation of nerve and that these grafts allowed substantial host axonal regeneration through them. These experiments will be extended to larger isografts as well as to allografts. It will be of interest to determine whether cryopreservation alters allograft antigenicity.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01-NS-02429-07 LNC</div>
PERIOD COVERED <div style="text-align: center; font-weight: bold;">October 1, 1985 through September 30, 1986</div>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <div style="text-align: center; font-weight: bold;">Coordinate Changes in Brain Energy Metabolism and Protein Synthesis</div>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="text-align: center;">             P. I. :    T.S. Nowak, Jr., Ph.D.    Senior Staff Fellow    LNC    NINCDS           </div>		
COOPERATING UNITS (if any)  <div style="text-align: center;">None</div>		
LAB/BRANCH <div style="text-align: center;">Laboratory of Neurochemistry</div>		
SECTION <div style="text-align: center;">Section on Cellular Neurochemistry</div>		
INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20892</div>		
TOTAL MAN-YEARS: <div style="text-align: center;">0</div>	PROFESSIONAL: <div style="text-align: center;">0</div>	OTHER: <div style="text-align: center;">0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews           </div> <div> <input type="checkbox"/> (b) Human tissues           </div> <div> <input checked="" type="checkbox"/> (c) Neither           </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <div style="text-align: center; padding-top: 20px;"> <p>This project has been transferred to LNNS.</p> <p>Publication:</p> <p>Nowak, T. S., Jr.: Synthesis of a stress protein following transient ischemia in the gerbil. <u>J. Neurochem.</u> 45: 1635-41 (1985).</p> </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-01586-19 LNC

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Trophic Interactions of Neuronal and Target Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I. : A. A. Zalewski, M.D. Section Head LNC NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Section on Neuronal Development and Regeneration

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.0

PROFESSIONAL:

0.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been terminated and the remaining aspects of it transferred to Project Number Z01-NS-02254-10 LNC. Further progress in identifying the trophic agent of sensory neurons responsible for taste bud formation and regeneration must await the development of a tissue culture model. To date, we have not been successful in culturing buds. We have, however, been able to establish the in vivo specificity of neurons and epithelium in forming buds.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01-NS-02254-10 LNC</b>
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Repair of Injured Nervous Tissue with Foreign Grafts*</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between;"> <span><b>P. I. : A. A. Zalewski, M.D.</b></span> <span><b>Section Head</b></span> <span><b>LNC</b></span> <span><b>NINCDS</b></span> </div>		
COOPERATING UNITS (if any) <b>None</b>		
LAB/BRANCH <b>Laboratory of Neurochemistry, IRP, NINCDS</b>		
SECTION <b>Section on Neuronal Development and Regeneration</b>		
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, Maryland 20892</b>		
TOTAL MAN-YEARS: <div style="text-align: center;"><b>3.5</b></div>	PROFESSIONAL: <div style="text-align: center;"><b>1.0</b></div>	OTHER: <div style="text-align: center;"><b>2.50</b></div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>Several studies were performed to evaluate factors which might lead to the use of foreign grafts to aid in the repair of injured nervous tissue. A comparative study was performed to determine whether the immunosuppressive agent <u>Cyclosporin-A</u> (Cy-A) prevented the rejection of xenogeneic (cross-species) neurons. The results demonstrated that mouse and hamster but not guinea pig neurons survived in grafts in Cy-A treated rats. However, no neurons survived in any other donor-host combination of those species (e.g., rat to hamster). The effect of Cy-A on xenografts is therefore unpredictable, and clinical implications drawn from data generated in a single donor-host combination must be guarded. In another study, we tried to determine the prime target tissue of the immune response directed towards a nerve allograft. We found that the <u>vascular system</u> was rejected first leading to infarction of the graft and the disappearance of Schwann cells from it. Another experiment was done to evaluate the status of the <u>blood-nerve barrier</u> in surviving nerve allografts in Cy-A immunosuppressed rats. The endoneurial graft vessels became permeable to intravenously injected horseradish peroxidase (HRP) during the acute phase of Wallerian degeneration, but they restored their normal impermeability following the remyelination of host axons that had regenerated through the graft. The perineurial-nerve barrier in allografts, as in normal nerve, remained impermeable to HRP that leaked from epineurial vessels throughout the period of graft survival. Other data revealed that in rats a nerve graft could be cryopreserved (i.e., frozen and the cells in it remain alive) in dimethyl sulfoxide and later used to repair injured nerve. This result indicates the feasibility of establishing banks of cryopreserved nerves since they function, as conduits for host axonal growth, better than frozen, killed grafts.</p> <p>*[Formerly "Repair of Injured Nerve With a Nerve Allograft"]</p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-00813-25 LNC

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Enzymological Aspects of Neural Functions

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I. :	R.W. Albers, Ph.D.	Chief, Sec. on Enzyme Chemistry	LNC	NINCDS
Others :	A.K. Hazra, M.D.	Visiting Associate	LNC	NINCDS
	A.S. Hobbs, Ph.D.	Expert Consultant	LNC	NINCDS
	P.M. Rowe, Ph.D.	Staff Fellow	LNC	NINCDS

## COOPERATING UNITS (if any)

J. P. Froehlich, NIA, NIH, Baltimore, MD  
R. H. Huang, Univ. Mo. Health Sci., Kansas City, MO  
T. S. Nowak, LNNS, NINCDS

## LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

## SECTION

Section on Enzyme Chemistry

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

3.9

## PROFESSIONAL:

2.2

## OTHER:

1.7

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project is comprised of research into the structure and functioning of ion transport systems. Major current studies are 1) research into the basis of the kinetic heterogeneity exhibited by the Na,K-ATPase as manifest in several different rapid-quenching experiments, 2) research into the basis of the structural heterogeneity manifest in the electrophoretic mobility of the catalytic subunit of the Na,K-ATPase from Electrophorus electricus organ, and 3) development and characterization of antibodies to synthetic peptides corresponding to selected portions of transport ATPase primary structure.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01-NS-02605-03 LNC
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanism of Membrane Biogenesis and Exocytosis *		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between; align-items: center;"> <span>P. I. : Stephen P. Chock, Ph.D. Expert</span> <span>LNC NINCDS</span> </div>		
COOPERATING UNITS (if any)  Elsa A. Schmauder-Chock, Department of Experimental Hematology, Armed Forces Radiobiology Research Institute (AFRRI)		
LAB/BRANCH Laboratory of Neurochemistry, IRP, NINCDS		
SECTION Section on Enzyme Chemistry		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 0.75	OTHER: 0.75
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>Until now, there has been no direct evidence on the mechanism by which a cell can generate membrane [Chock and Schmauder-Chock (1985) <i>Biochem. Biophys. Res. Comm.</i> 132, 134-139]. This project, while set out to unravel the mechanism of secretion, has uncovered one of the most fundamental processes in biology -- membrane biogenesis and membrane fusion. It not only offers a new perspective on how a cell can rapidly assemble new membrane from stored membrane precursor elements, it also resolves many unanswered questions concerning exocytosis. This project therefore, spans two very fundamental areas: membrane, without it cellular life would be impossible; and <u>exocytosis</u>, the mechanism by which cells secrete, communicate, and regulate. The significance of this project to biomedical research and the mission of the Institute is self-evident. This project has now been terminated at NIH and is being conducted at AFRRI.</p> <p>In the past year, we have established our hypothesis of <u>de novo membrane generation</u> on much firmer ground by accomplishing the following: (1) We have demonstrated by serial sections and by the principle of metachromasy that the extensive expansion of secretory perigranular membrane can occur prior to its fusion with the plasma membrane. This set the stage for the requirement of the occurrence of a rapid new membrane assembly and membrane insertion into the perigranular membrane as part of the mechanism for granule exocytosis. (2) We have succeeded in isolating quiescent secretory granules from rat mast cells and have determined their phospholipid contents. The results indicate that the granule contains enough phospholipid alone to quadruple the surface area of the perigranular membrane. (3) We have found evidence that new membrane assembly also occurs in A23187 induced mast cell granule exocytosis. (4) Preliminary experiments on antigen challenge of IgE-p sensitized mast cells also show the occurrence of <u>de novo membrane generation</u>, suggesting that <u>this rapid process of membrane biogenesis is an integral part of the mechanism of exocytosis.</u></p> <p>*[Formerly: "Mechanism of Mast Cell Secretion &amp; Membrane Generation"]</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02701-01 LNC

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Quantitative Studies of Nerve Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I. : R.W. Albers, Ph.D. Chief, Section on Enzyme Chemistry LNC NINCDS

COOPERATING UNITS (if any)

Lloyd Guth, M.D., Department of Anatomy, University of Maryland School of Medicine

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Section on Enzyme Chemistry

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.25

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

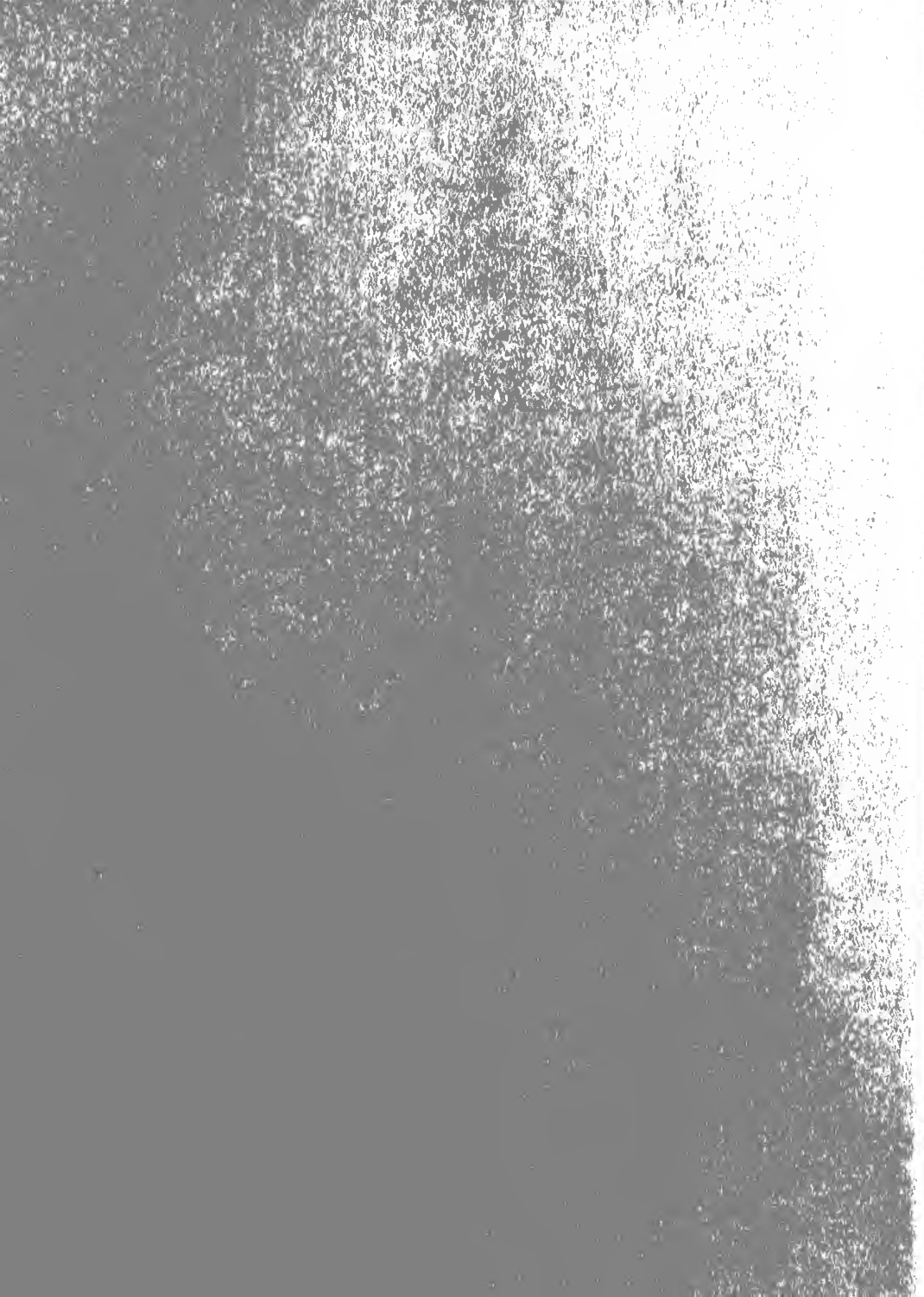
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

A collaborative project on quantitative studies of nerve regeneration has been initiated. Nerve regeneration can be logically considered in 4 aspects: 1) the peripheral signal; 2) the neuronal growth response; 3) axonal guidance; and 4) termination. Current work is directed at quantitatively evaluating the neuronal growth response in peripheral nerves. To this end we are using neurofilament protein as a neuron-specific marker. We have demonstrated a proximal to distal gradient of phosphorylated NFP in normal sciatic nerves. Nerve crush or transection is followed by depletion of distal NFP and alterations in the normal gradient of NFP proximal to the lesion. This and other markers of neuronal function are being used to investigate the events that occur during the regenerative process and to assess the effects of various experimental regimes.









# ANNUAL REPORT

October 1, 1985 through September 30, 1986

## Laboratory of Neuro-otolaryngology

National Institute of Neurological and Communicative Disorders and Stroke

### Table of Contents

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Z01NS02217-11 LNO	



# ANNUAL REPORT

October 1, 1985 through September 30, 1986  
Laboratory of Neuro-otolaryngology, IRP  
National Institute of Neurological and  
Communicative Disorders and Stroke

Jürgen Fex, M.D., Ph.D., Chief

The Laboratory has continued to provide new knowledge within the framework of its two Projects: Project Number ZO1NS02216-11 LNO, Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis and Project Number ZO1NS02217-11 LNO, Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus. We aim at a better understanding of how the inner ear translates sound waves into nerve activity and how the cochlear nucleus processes the auditory information that it receives from the inner ear.

Our findings of last year's Annual Report, that isolated outer hair cells when electrically stimulated give responses that are not dependent on metabolically based energy, have since then been complemented with more experiments, analyzed, and submitted for publication. The study is now in print, in Nature. Our initial interpretation was upheld, that the evidence is excellent for an electro-osmotic mechanism as explanation for the shape changes of outer hair cells that we saw. Outer hair cells make one of two mechanosensitive hair cell populations in the mammalian cochlea and their role in hearing may be to modulate the micromechanical properties of the hearing organ through a mechanical feedback mechanism. The type of phenomenon we have described may be importantly involved in the function of outer hair cells as well as of other cells or cell components, such as dendritic spines where the structural configuration may provide conditions for electro-osmotic driven shape changes. We will continue to carry out experiments on isolated outer hair cells, seeking to establish the structural basis and the mechanism of electrically evoked mechanical changes in such cells. The structure of hair cell stereocilia and their interaction with the tectorial membrane in the organ of Corti, or with the gelatinous membrane and otoconia crystals in otolith organs, will be studied using video enhanced light microscopy and rapid freezing electron microscopy.

The number of hair cells and the whole auditory organ keep growing during most or all of the life time of the shark and the fish and the toad. Dr. J. T. Corwin has studied this remarkable phenomenon during several years. We have recently established collaboration with Dr. Corwin, for studies aiming at an understanding of how this ever continuing growth of the hearing organ is programmed and how it may be controlled. If reached, such an understanding could be a basis for a successful treatment of sensory hair cell damage in the human sensory organ. As a start in this common undertaking we are trying to produce markers, of membranes and other cell components of cells of different populations and different levels of differentiation, in auditory sensory organs. We have carried out a series of immunocytochemical experiments, trying to mark putative ACh receptors on outer hair cells with a

fluorescent bungarotoxin conjugate. We have also used anti-ACh-receptor antibodies. We have tried to find GABA-receptors on outer hair cells with anti-GABA-receptor antibodies; antibodies of other specificities have also been used. We have not yet seen labeling in these experiments and are modifying our labeling assays for increase of sensitivity. In our search for labels, and for other purposes, we are also initiating experiments for producing monoclonal antibodies, at first using cells of auditory sensory organs of the shark as carriers of antigens.

A very recently initiated study is in progress in which we seek to purify and characterize the enzyme for force generation during intracellular organelle translocation, with microtubules prepared from bovine brain and translocator proteins from cultured Acanthamoeba. Similar procedures will later be used to purify and characterize translocator proteins from neuronal cells.

Our Laboratory has been studying neurotransmitters in the auditory system also in order to identify the neurotransmitters of major neuronal pathways and determine how changes in the system affect the expressions of neurotransmitters, enzymes and receptors of these pathways. Developing specific antibodies and using immunocytochemistry, we have made enormous progress in the characterization of amino acid neurotransmitters, providing new information on their general properties. For example, our recent work on glycine and the glycine receptor has helped to develop an immunocytochemical marker for glycine as neurotransmitter which can be used throughout the nervous system.

The general findings on the distribution of the glycine receptor were presented in last year's Annual Report and in two publications. This past year the glycine receptor has been characterized in the cochlear nucleus with two monoclonal antibodies called Gly rec 2 and Gly rec 7. Gly rec 2 binds to the ligand binding subunit of the receptor while Gly rec 7 binds to a nonligand binding subunit. These antibodies showed similar distributions in the cochlear nucleus, as seen under the light microscope. Immunoreactive labeling was seen throughout the cochlear nucleus, in the ventral cochlear nucleus often as puncta around the perimeter of cell bodies. Gly rec 7 was found to tolerate low levels of glutaraldehyde. With this antibody the glycine receptor distribution was studied with electron microscopy throughout the ventral cochlear nucleus. Labeling was present on the cell body of every cell type found, except for cells identified as stellate cells, which were labeled on dendrites only. Of the several synapses previously described, immunoreactivity was found to be often associated with the population of synapses containing flattened synaptic vesicles. However, particularly in the posteroventral cochlear nucleus, labeling may also be present at synapses containing oval synaptic vesicles.

As we have shown, glycine receptor immunoreactivity in the cochlear nucleus is widespread. We have begun to look for the origins of the corresponding putative glycinergic inputs, using antibodies against glycine, which would be expected to label cell bodies and presynaptic terminals of glycinergic neurons. With these antibodies four

immunoreactive populations of cells were found: cells in the superficial layer of the dorsal cochlear nucleus, cells in the deep layer of the dorsal cochlear nucleus, and large and small cells in the ventral cochlear nucleus.

The glycine immunoreactive cells in the superficial dorsal cochlear nucleus were similar to cells labeled with anti-GABA antibodies. Double labeling studies showed that these cells were labeled with both anti-GABA and anti-glycine antibodies.

Using double labeling studies with retrograde tracers and anti-glycine antibodies, we will determine if the glycine-immunoreactive large cells in the ventral cochlear nucleus are giant cells that project to the contralateral cochlear nucleus. If that is so, anterograde tracers combined with anti-glycine receptor antibodies will be used to strengthen the argument that this pathway is glycinergic. The pathway may be a major inhibitory input to the cochlea nucleus and an ideal system for defining and studying glycine postsynaptic receptors.

As reported during previous years, we have provided good evidence towards the hypothesis that a major transmitter of the auditor nerve is an excitatory amino acid. During this past year we have begun a project to biochemically characterize excitatory amino acid receptors, initially studying the effects of various detergent treatments on the binding of  $^3\text{H}$ -glutamate and  $^3\text{H}$ -kainate to rat brain membrane. We were able to solubilize kainate binding activity. We will now determine whether or not the solubilized binding site is pharmacologically similar to the membrane associated kainate receptor. We will then work on purifying the binding protein(s). Wheat germ agglutinin binding will give some purification, but we will try to design an affinity column. We expect to eventually effectively purify the receptor by using monoclonal antibodies made against a partially purified preparation.

In collaboration with the Laboratory of Molecular Biology, NINCDS, we used our two affinity purified anti-glutamate dehydrogenase (anti-GDH) polyclonal antibodies to detect a bacteriophage expressing an immunoreactive recombinant protein. A comparison of peptides from this protein and from bovine GDH strongly suggested that the bacteriophage clone encoded part of the GDH gene. We will continue efforts to obtain cDNA clones for the other important enzymes in glutamate metabolism. When we have such clones we will measure levels of mRNA in cells and tissue, thereby confirming and extending our immunocytochemical studies on these enzymes.

We have begun to use neurofilament proteins of auditory nerve components (spiral ganglion cells, central and peripheral processes) as parameters to follow nerve changes after lesions of auditory sensory cells in the organ of hearing. This is a new study and we have thus far concentrated on developing techniques for dissection, micro gel electrophoresis and immunocytochemistry of cochlear samples. Primary immunoblot analysis of gels of the auditory nerve show the presence of

the three neurofilament subunits. The goal of this research is to identify critical auditory nerve proteins and characterize them under normal and abnormal conditions. One of our major interests is to determine biochemical changes that occur in the auditory nerve after hair cell loss. In previous work we have identified two glycoproteins in the auditory nerve whose expressions are significantly altered by the loss of hair cells. We are analyzing neurofilament proteins to determine if this group is also changed by hair cell loss; in other systems, analogous insults have been reported to alter the expression of neurofilament subunits.

We reported last year that the Laboratory was coming below a minimal critical mass of personnel. In September 1985, our situation became even worse through the completely unexpected and sudden loss from the Laboratory of a key investigator. On the other hand, this unexpected situation proved an excellent opportunity to increasingly direct the Laboratory research efforts towards basic cell biology issues. And, fortunately, we were generously supported in recruiting new people. Our recruiting efforts have been very successful, although at the price of considerable expenditure of time. If our present plans come through, the Laboratory should by the beginning of the next calendar year not be uncomfortably short of personnel. By then we expect to have on board at least an additional Staff Fellow, and an additional Visiting Fellow/Visiting Associate. By then we also expect to have replaced, or recruited a replacement for, the Biologist at the Laboratory who now says she will leave at about September 1, 1986 (this Report is submitted on July 15, 1986).



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02216-11 LNO

## PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jörgen Fex	Chief	LNO, IRP, NINCDS
Others:	B. Kachar	Visiting Associate	LNO, IRP, NINCDS
	N. Najam	Senior Staff Fellow	LNO, IRP, NINCDS
	M. Tachibana	Visiting Scientist	LNO, IRP, NINCDS
	M. J. Frye	Electronics Technician	LNO, IRP, NINCDS
	M. H. Parakkal	Biol. Lab. Technician	LNO, IRP, NINCDS
	K. A. Reeks	Biologist	LNO, IRP, NINCDS

COOPERATING UNITS (if any) Lab. of Biophysics, NINCDS (K. Iwasa); Lab. of Cell Biol., NHLBI (H. Fujisaki and J. P. Albanesi); Kresge Hear. Res. Inst., Univ. Michigan, Ann Arbor, MI (R. A. Altschuler); The Johns Hopkins Univ., Baltimore (W. L. Brownell); Békésy Lab. Neurobiol., Univ. of Hawaii, Honolulu, HI (J. T. Corwin).

## LAB/BRANCH

Laboratory of Neuro-otolaryngology

## SECTION

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

3.9

## PROFESSIONAL:

2.1

## OTHER:

1.8

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project provides new knowledge of the auditory mechanisms of the inner ear through studies of the biochemistry, morphology, pharmacology and physiology of auditory sensory cells, supporting cells and neurons of the inner ear, using small mammals, bullfrogs and sharks. An immunocytochemical study of receptors in the cochlea is in progress. Immunological studies have been initiated to provide specific markers of cells of the organ of Corti. A study is in progress in which we seek to purify and characterize the enzyme for force generation during intracellular organelle translocation, preparing microtubules from bovine brain and translocator proteins from cultured Acanthamoeba.

The possibility that outer hair cells may modulate the micromechanical properties of the hearing organ through mechanical feedback mechanism led us to the following study, now in press in Nature. Isolated outer hair cells were prepared from the organ of Corti of guinea pig by non-enzymatic mechanical dissociation. Sinusoidal potential gradients were passed across such hair cells, causing oscillatory elongation and shortening of the outer hair cells and oscillatory movements of intracellular organelles as was directly visualized and measured from the video recorded images. Maximal responses were obtained when cells were longitudinally aligned with the direction of the electric field. The responses were unaffected by incubation of the cells with medium containing inhibitors of ATP production (dinitrophenol and iodoacetic acid). Responses were enhanced by lowering the ionic concentration. Cells with little movement in the standard medium reversibly showed robust movement after ionic dilution. Based on these experimental findings we proposed a novel mechanism for mechanical changes in outer hair cells based on an electro-osmotic effect and not dependent on conventional contractile processes.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02217-11 LNO																		
PERIOD COVERED October 1, 1985 to September 30, 1986																				
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus																				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: R. J. Wenthold</td> <td style="width: 33%;">Chemist</td> <td style="width: 33%;">LNO, IRP, NINCDS</td> </tr> <tr> <td>Others: D. R. Hampson</td> <td>Staff Fellow</td> <td>LNO, IRP, NINCDS</td> </tr> <tr> <td>J. Dau</td> <td>Visiting Fellow</td> <td>LNO, IRP, NINCDS</td> </tr> <tr> <td>D. Huie</td> <td>Chemist</td> <td>LNO, IRP, NINCDS</td> </tr> <tr> <td>M. H. Parakkal</td> <td>Bio Lab Tech (Micro)</td> <td>LNO, IRP, NINCDS</td> </tr> <tr> <td>K. A. Reeks</td> <td>Biologist</td> <td>LNO, IRP, NINCDS</td> </tr> </table>			PI: R. J. Wenthold	Chemist	LNO, IRP, NINCDS	Others: D. R. Hampson	Staff Fellow	LNO, IRP, NINCDS	J. Dau	Visiting Fellow	LNO, IRP, NINCDS	D. Huie	Chemist	LNO, IRP, NINCDS	M. H. Parakkal	Bio Lab Tech (Micro)	LNO, IRP, NINCDS	K. A. Reeks	Biologist	LNO, IRP, NINCDS
PI: R. J. Wenthold	Chemist	LNO, IRP, NINCDS																		
Others: D. R. Hampson	Staff Fellow	LNO, IRP, NINCDS																		
J. Dau	Visiting Fellow	LNO, IRP, NINCDS																		
D. Huie	Chemist	LNO, IRP, NINCDS																		
M. H. Parakkal	Bio Lab Tech (Micro)	LNO, IRP, NINCDS																		
K. A. Reeks	Biologist	LNO, IRP, NINCDS																		
COOPERATING UNITS (if any) Laboratory of Molecular Biology, IRP, NINCDS (C. Banner, L. Vitkovic, S. Silverman); Kresge Hearing Research Institute, University of Michigan, Ann Arbor (R. A. Altschuler); Center for Molecular Biology, University of Heidelberg, Heidelberg, Federal Republic of Germany (H. Betz).																				
LAB/BRANCH Laboratory of Neuro-otolaryngology																				
SECTION																				
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																				
TOTAL MAN-YEARS: 5.8	PROFESSIONAL: 2.6	OTHER: 3.2																		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )  <p>This multifaceted project provides new knowledge of how the cochlear nucleus processes the information that it receives from the inner ear through the auditory nerve. Neurotransmitters, neuromodulators, related enzymes and receptors active at major synapses in auditory nuclei in the brainstem are identified and characterized. It is determined how the auditory nerve and the cochlear nucleus may change after lesions in the cochlea. Small mammals have been used in these studies.</p> <p>I. Our results show, in <u>immunocytochemical</u> studies, with <u>light</u> and <u>electron microscopy</u>, using <u>monoclonal antibodies</u> against the <u>glycine receptor</u> and <u>polyclonal antibodies</u> against GABA and against <u>glycine</u>, that putative GABAergic and glycinergic innervation is widespread in the <u>auditory brainstem</u>. In <u>double labeling</u> studies, cells in the superficial dorsal cochlear nucleus showed immunoreaction to both anti-GABA and anti-glycine antibodies.</p> <p>II. We have begun to biochemically characterize <u>excitatory amino acid receptors</u> by studying how the binding of <u>radioactively labeled glutamate</u> and <u>kainate</u> changes with various detergent treatments of rat brain membrane.</p> <p>III. In collaboration with the Laboratory of Molecular Biology, NINCDS, we used our two <u>affinity purified anti-glutamate dehydrogenase</u> (anti-GDH) <u>polyclonal antibodies</u> to detect a bacteriophage expressing an immunoreactive recombinant protein. A comparison of peptides from this protein and from bovine GDH strongly suggested that the <u>bacteriophage clone</u> encoded part of the <u>GDH gene</u>.</p> <p>IV. We have begun to use <u>neurofilament proteins</u> of <u>auditory nerve</u> components (spiral ganglion cells, central and peripheral processes) as parameters to follow <u>nerve changes</u> after <u>lesions</u> of auditory sensory cells in the organ of hearing.</p>																				





# ANNUAL REPORT

October 1, 1985 through September 30, 1986

Laboratory of Neuropathology and Neuroanatomical Sciences  
National Institute of Neurological and Communicative Disorders and Stroke

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# ANNUAL REPORT

October 1, 1985 through September 30, 1986

Laboratory of Neuropathology and Neuroanatomical Sciences, IRP  
National Institute of Neurological and Communicative  
Disorders and Stroke

Igor Klatzo, M.D. Chief

The Laboratory of Neuropathology and Neuroanatomical Sciences (LNNS) has continued its general program focussed primarily on elucidation of various mechanisms operative in pathophysiology of cerebral ischemia and brain edema, with the idea that such studies may provide a basis for designing proper therapeutic measures for treatment of these conditions. The presence of common features in pathophysiology of cerebral ischemia and epileptic seizures provided reason for extension of our studies to certain aspects, common to both conditions, such as disturbance in cerebrovascular permeability.

The Section of Cerebrovascular Pathology has continued further investigations on dynamics and mechanisms operative in post-ischemic brain edema.

It has been generally assumed until now that in ischemic brain injury, the developing edema is of the cytotoxic type and that only in later stages the vasogenic edema makes its appearance, in association with severe destruction of brain parenchyma. Contrary to such assumptions, our recent studies have indicated that in the temporary cerebral ischemia, changes in cerebrovascular permeability play from the beginning a paramount role and that early vasogenic edema may significantly influence the final outcome of ischemic injury.

The vasogenic character of an early post-ischemic edema became evident from our most recent observations in cats subjected to 20 min of the temporary middle cerebral artery (MCA) occlusion. In these animals, evaluation of the electrical cerebral impedance (ECI) from the caudate, subjected to ischemia, revealed, that after the marked elevation of ECI during the ischemic occlusion, the release of occlusion resulted in a precipitous drop in ECI, following which two groups of animals could be recognized: A. Cats, in which ECI returned to the normal baseline levels, showed, when sacrificed at 6 hours of recirculation, no evidence of blood-brain barrier (BBB) leakage, nor increase of water content in the caudate subjected to ischemic insult. B. Cats, in which ECI following

recirculation fell to below baseline values, revealed, when sacrificed after 6 hours, evidence of abnormal permeability of BBB and a significant edema in the exposed to ischemia caudate. The vasogenic character of this edema is implied on the basis of correlation between increment in water content, associated with increased permeability of the BBB, and CEI drop below normal base values, indicating widening of extracellular spaces, which is the main criterion of vasogenic edema.

The significance of the early barrier opening associated with extravasation of serum proteins on the further post-ischemic course of ischemic injury has been elucidated in our recent study dealing with prevention of reactive hyperemia by hypovolemia, produced by withdrawal of blood just before the release of MCA occlusion.

Our observations indicated that in cats, sacrificed 3 hrs after release of MCA occlusion, the ischemic sites, associated with reactive hyperemia, showed evidence of BBB breakdown to proteins and significantly more severe edema than the sites, subjected to similar in intensity ischemia, but without reactive hyperemia, and which failed to reveal leakage of Evans Blue (EB) tracer. In cats sacrificed at 3 and 14 days, the ischemic sites, which showed reactive hyperemia, revealed much more severe ischemic brain tissue injury than was observed at the sites without reactive hyperemia and no evidence of any EB leakage. These studies thus strongly suggest that reactive hyperemia, which follows release of a major cerebral artery occlusion, plays a significant role in the breakdown of the BBB to proteins and in increasing the severity of post-ischemic edema and of ischemic brain tissue injury. Since prevention of reactive hyperemia by hypovolemic withdrawal of blood would be difficult to apply in clinical situations, we are currently investigating a possibility of preventing hyperemia by pharmacological action of theophylline administered at the time of release of MCA occlusion. The preliminary results appear to be promising, showing a significant suppression of reactive hyperemia and significantly lesser ischemic edema in animals sacrificed after 3 hours.

The presence of common features in pathophysiology of cerebral ischemia and epileptic seizures drew our interest to explore certain aspects of the latter condition. One of these aspects has concerned the BBB disturbances, which occur in both disorders.

Our previous studies in rabbits on BBB and cerebral blood flow (CBF) changes in acute hypertension induced by injection of adrenalin or metaraminol and in bicuculline-induced seizures revealed that in acute hypertension, the breakdown of the BBB was clearly related to the rate of mean arterial blood pressure (MABP) rise, being much less pronounced in the metaraminol injected animals, which showed much slower blood pressure



elevation rate. Otherwise, in acute hypertension, the BBB leakage to EB tracer was observed in regions which also showed the highest elevations of CBF. In bicuculline-induced seizures the BBB leakage was present only in animals in which MABP rose about 50mm Hg with the onset of convulsive motor activity. On the other hand, there was no evident correlation between the amplitude of CBF rises and EB extravasations, and in some regions showing very high rCBF elevation the BBB remained intact. Our recent ultra-structural observations in bicuculline-induced seizures, using horse-radish peroxidase (HRP), revealed that the passage of this protein tracer in the areas of increased BBB permeability took place primarily by pinocytotic transport. After the HRP had reached the neuropil, it accumulated in the interstitial spaces and penetrated synaptic clefts. Uptake of the tracer in vesicular form into the presynaptic boutons, presumably excitatory ones, was observed in all brain regions showing the increased vascular permeability.

Our studies on vascular permeability changes in epileptic seizures thus suggest that released neurotransmitters, involved in vascular autoregulation, may be taken up by greatly activated neuronal structures and this may further influence regional transmitter milieu in brain tissue.

Transitory ischemic attacks (TIAs), with their prodromal symptomatology frequently leading to a stroke, present some of the most important clinical problems among cerebrovascular disorders. Their epidemiological frequency and potential for a permanent, incapacitating brain injury has fueled immense clinical interest and investigations for many years, the main rationale being that proper management and treatment could prevent serious brain damage in a great number of the cases. These extensive clinical investigations, strangely, have been receiving scarcely any support from experimental studies, and generally these attempts have been faulted by poor reproducibility and lack of systematic assessment of various parameters of ischemic injury.

The experimental TIA model developed in gerbils in our laboratory represents the first model which can be used in an easy, reproducible way to study systematically various aspects concerning the effect of repeated ischemic attacks upon brain tissue. The experimental design is based upon surrounding both common carotid arteries at the neck by soft plastic sleeves which contain inside two systems of threads, responsible for compressing or releasing compression of the carotid arteries. The implantation of the sleeves takes place 7 - 10 days prior to TIA experimentation, leaving free ends of threads outside. A TIA is produced by pulling the compressing threads for any desired period of time; the release of carotid occlusion is achieved by pulling on the set of releasing threads. To carry out individual TIAs requires minimal anesthesia, since it is virtually free of trauma and pain.

Our preliminary observations using this model can be summarized as follows: 1) Three TIAs lasting 5 minutes each, induced at 1 hour intervals during the period of hypoperfusion, result in progressively greater drops of CBF during occlusion, and progressively lower flow levels during the period of hypoperfusion. 2) Three 5 minute TIAs induced at 1 hr intervals resulted in severe edema of the subjected to ischemia brain structures, when assessed after 24 hours of recirculation. This edema, measured by specific gravity, was significantly more severe than that resulting from a single 15 minute occlusion, whereas gerbils with a single 5 min ischemia showed full resolution of edema after 24 hours. 3) Morphological observations on the cerebral cortex, caudate and hippocampus revealed histological picture of severe edema in gerbils subjected to 3 x 5 min occlusion at 1 hour intervals when sacrificed at 24 hours. The edematous changes were much more pronounced than in gerbils after the single 15 min occlusion. No evidence of edema was seen in animals subjected to a single 5 min occlusion. 4) The cumulative effect of repeated ischemic insults appears to correlate with the state of hypoperfusion, which approximately for 6 hours follows, the release of carotid occlusion. No cumulative effect was observed in animals with three 5 min occlusions spaced at 3 min and 24 hour intervals, which are not associated with hypoperfusion.

Recently, the potential for application of various neurochemical approaches has been widened by Dr. T. Nowak joining the Section and his preliminary observations on protein synthesis changes in TIAs, using an in vitro ribosome, run off assay, indicated that 3 x 5 min occlusions at 1 hr interval resulted in significantly lower protein synthesis activity at 2 and 4 hours after the last TIA than those found at 2 and 4 hours following a single 5 min occlusion.

A considerable attention of the Section of Cerebrovascular Pathology has been directed to studies on ischemic penumbra.

A severe, regional, ischemic insult, as a rule, results in a center of complete brain tissue destruction, which is surrounded by extensive, less severely affected areas retaining potential for eventual recovery. Such a pattern of injury is supported by common clinical observations indicating that severely incapacitated stroke patients can retrieve a remarkable amount of previously lost functions. Descriptively, in analogy to the half-shaded zone around the center of a complete solar eclipse, this part of the ischemic brain capable for recovery has been termed "penumbra". Since it is impossible to restore brain tissue irreversibly destroyed by ischemia, there is a strong rationale and motivation for research focussed on twilight zones of ischemic penumbra, where proper application of therapeutic measures could induce and facilitate neuronal recovery and restitution of function.

Originally, penumbra has been described as the condition of the ischemic brain with blood flow between two thresholds--the upper threshold of electrical failure and the lower of energy failure and ion pump failure leading to irreversible neuronal death. In our own studies, we apply a broad concept of penumbra which encompasses conditions involving both global and regional cerebral ischemia, the main criterion being the retention of ability for recovery in even severely injured neurons.

In our current studies on penumbra, a twilight zone surrounding the infarcted tissue is reproduced by applying insult at the threshold levels of ischemic injury, which may either resolve into almost complete recovery, or succumb to irreversible damage of the brain tissue. As the experimental model we are using 10 - 20 minute occlusion of the middle cerebral artery (MCA) in the cat, in which various parameters of ischemic injury are being investigated. Since an important indicator of viability of the neurons is their protein synthesis our studies on penumbra utilize methods of incorporation in vivo of 3H-labeled aminoacids at various post-ischemic periods, as well as evaluation of ribosomal aggregation in neuronal cytoplasm. Generally, a considerable effort in our studies on penumbra is directed to determination of factors which could be manipulated to improve an outcome of ischemic injury.

Changes in brain energy metabolism and protein synthesis have been studied following transient bilateral ischemia in the gerbil. Project areas include: a) continued characterization of the stress or heat shock response following transient ischemia; b) evaluation of mechanisms responsible for the ischemia-induced depletion of hippocampal dynorphin; and c) mechanisms of overall protein synthesis inhibition and recovery following ischemia.

The initial demonstration of increased translation of the major mammalian stress protein, hsp 70, is being extended to the transcriptional level. Preliminary results demonstrate the induction of mRNA's for hsp 70 and ubiquitin, using rat cDNA probes, following hyperthermia in the gerbil, and the effects of ischemia can now be investigated. Immunohistochemical localization of hsp 70 is being pursued with monoclonal antibodies specific for the induced protein.

An earlier observation of dynorphin A depletion in gerbil brain following ischemia has been localized exclusively to hippocampus. The time course of changes in peptide levels is consistent with increased activity of the dynorphin-containing dentate granule cells following ischemia. These results extend the concept of hippocampal vulnerability to the neurochemical level, and suggest a role for the intrinsic hippocampal circuitry in proposed excitotoxic mechanisms for loss of hippocampal CA1 neurons following ischemia.

The Section on Cerebrovascular Pathophysiology has continued its study of the effects of temporary focal ischemia of the cat brain upon the cerebral extracellular space (CES). Attention was given to examining the correlation of edematous changes and evidence of leakage through the blood-brain barrier by tracers and with changes in the (CES) associated with middle cerebral artery occlusion (MCAO) for periods of time ranging from 5 minutes to 3 hours. The changes in CES were determined by measurement of changes in cerebral electrical impedance (CEI) using a platinum microelectrode array implanted in the cortex and/or the caudate nucleus. Edematous changes were based on differences in water content determined by the gravimetric method. Leakage through the blood-brain barrier was assessed with sodium fluorescein and/or Evans Blue. The regional blood flow rCBF was determined by hydrogen clearance method using one of the electrodes in the array.

Particular attention was given to the recovery phase after 20 minutes of occlusion of the middle cerebral artery, with the rCBF below 12 ml/100g/min. Release of the occlusion was promptly followed by marked reactive hyperemia, significantly higher in the caudate than in the cortex even though the rCBF flows were similar. The hyperemias high levels of hyperemia correlated with increased permeability of the BBB to NaFl.

The CEI in one group of animals, recovered to normal pre-occlusion values. It showed no edema. The remaining animals revealed a vasogenic character by a decrease of the CEI below the base/control level and a specific gravity lower than normal signifying edema present. Following a refractory period, a second opening in the barrier to NaFl was observed at 24 hrs only in the caudate. These studies revealed a correlation between the intensity of hyperemia, opening of the BBB, edema and damage to neurons. A remarkable degree of recovery was often observed in morphological appearance of neurons in the cortex and in the caudate two weeks after recirculation.

The continuous goals of the Section on Neurocytobiology have been: I) to develop and utilize new model systems for the investigation of basic mechanisms operative on the level of normal and pathologically altered blood-brain barrier (BBB) and cerebral blood flow (CBF): II) to study the metabolic processes occurring in cerebral ischemia and ischemic edema, especially their prevention and therapy.

I. During the last years both the recently established pure muscle cell culture (Spatz et al. Brain Res. 280: 387-391, 1983) and the previously developed endothelial culture derived from dissociated cerebral microvessels (Spatz et al. Brain Res. 191: 577, 1980) have been very useful

models for the continuous studies of cerebrovascular function related to the BBB, CBF and SBP.

Four different aspects related to the cerebral capillary function in vivo have been investigated in the in vitro models using the pure cerebrovascular endothelial and/or smooth muscle cell culture: a) Enzymic barrier, b) Prostaglandin receptors linked to AC activity, c) Effects of forskolin on cellular growth and morphology, and d) Interaction between cerebral capillary endothelium and immune cells.

a) The activity of catechol-O-methyltransferase (COMT) was investigated in cultured and propagated cerebromicrovascular endothelial and smooth muscle cells using high pressure liquid chromatography and immunocytochemistry. The existence of COMT was detected in both cell types. The immunocytochemical detection of COMT in both cell types is of particular interest since the reactivity to anti-COMT was previously undetected in the cerebral microvessels and absent from the smooth muscle cells of aorta and coronary vessels.

The demonstration of this enzyme activity in the cerebromicrovascular smooth muscle cells, in addition to the endothelium, indicates that the enzymatic barrier to catecholamine is not limited to capillaries, the main constituents of the blood-brain barrier.

b) The effect of prostaglandins ( $\text{PGE}_1$ ,  $\text{PGE}_2$ ,  $\text{PGF}_{1\alpha}$ ,  $\text{PGF}_{2\alpha}$ ,  $\text{PGD}_2$ ) on adenylate cyclase (AC) was investigated in cerebromicrovascular smooth muscle and glial cell cultures.  $\text{PGE}_1$  and  $\text{PGE}_2$ , in contrast to  $\text{PGF}_1$  and  $\text{PGF}_2$ , stimulated cAMP formation in both cell types. The smooth muscle AC system showed a greater affinity for E-type prostaglandins than the AC system in glial cells. Most importantly, a dose-dependent  $\text{PGI}_2$  but not  $\text{PGD}_2$  enhancement of cAMP formation was manifested in smooth muscle while a reverse AC reactivity of  $\text{PGI}_2$  and  $\text{PGD}_2$  was seen in glial cells. These findings indicate that the prostaglandin receptors linked to AC are distinct in each cell type.

c) The role of cyclic nucleotides in growth control and differentiation is yet unresolved. The recent availability of forskolin, a drug which directly stimulates the catalytic subunit of adenylate cyclase, allowed to study growth regulation of brain cells by cAMP from a new viewpoint. Separately cultured cerebromicrovascular endothelium smooth muscle and glial cells served as in vitro models for these studies. Forskolin reduced thymidin incorporation in all three cell types.

Exposure to forskolin led also to reversible drastic and immediate changes of cell morphology and F-actin composition in endothelium and smooth muscle. In glial cells, morphologic changes were visible only after

exposure to forskolin for more than 24 hours. These changes were accompanied by increased staining with antibodies against glial fibrillary acidic protein.

These findings support a role of cAMP in growth regulation of these cells and indicate that forskolin might be used as a tool to induce growth arrest and eventually differentiation in cell cultures from mammalian brain.

d) The collaborative studies with Drs. McCarron and McFarlin (Neuro-immunology Branch) have shown that freshly isolated cerebral endothelial cell (EC) lack Ia molecules and are incapable of presenting antigen. However, the expression of Ia on the surface of the EC can be induced by Con-A conditioned media and subsequently the EC are capable of presenting antigen. The antigen presentation by cerebral EC could be inhibited by anti-IA antisera syngeneic for cerebral EC donor haplotype. Most importantly, endothelial cells isolated from SJL mice with active experimental allergic encephalomyelitis were found to express I-A antigens and effectively present myelin basic protein (MBP) to sensitized T lymphocytes. In addition, immune cells cultured with mitogens or MBP release soluble factors which could induce I-A molecule on endothelial cells from normal mice and these cells acquired the capacity to present antigen to the same degree as EC from mice with EAE.

The expression of I-A molecules on cerebral vascular endothelium which normally do not express detectable levels of class II molecules may result in interaction with immune T cells and pathogenic reactions which occur in certain brain diseases which are associated with T cells such as MS and EAE.

II. The continuous studies of cerebral ischemia, its pathophysiology, prevention and therapy have been focussed on the ischemic effect on the metabolic pathway of 5-HT and its relationship to the development and prevention of brain edema.

Serotonin (5-HT) is both a central neurotransmitter and vasoactive agent. This monoamine exerts a wide range of metabolic and vascular effects on the cerebral circulation so it may well be involved in the mechanisms that link neuronal activity to cerebral blood flow.

a) Our previous results demonstrated that the ischemic edema is associated with an increased release of 5-HT and kinetic changes in the properties of synaptosomal serotonin ( $S_2$ )-receptor binding sites. In another study, we have also shown that either preischemic or postischemic treatment of gerbils with  $\gamma$ -hydroxybutyrate (GHB) endogenous central nervous system depressant reduces the formation of edema and stabilizes the 5-HT metabolism. To shed some more light on the pathomechanisms of

edema formation, we investigated the preischemic GHB effect on  $S_2$ -receptor binding sites.

These studies have shown that GHB given prior to the induction of bilateral carotid artery occlusion in gerbils prevented the ischemically induced changes in  $S_2$ -receptor binding sites using 3H-ketanserin as ligand (which labels specifically  $S_2$ -receptor sites). Thus, the capability of GHB to reduce the formation of ischemic edema and stabilizes the 5-HT metabolism (observed in previous studies) as well as to prevent ischemic changes in the kinetic properties of  $S_2$ -receptor binding sites reinforces the contention of GHB effect on 5-HT metabolic pathway.

b) Recently, we had demonstrated that the changes in the cerebral cortical content of 5-HT, its precursor tryptophan and its main metabolite 5-hydroxyindole acetic acid, unlike those of energy metabolites, depend on the age of the animal and the duration of ischemia. To shed some more light on this phenomenon, we studied the effect of ischemia on synaptosomal uptake and release of 5-HT in the cerebral cortex, hippocampus and striatum of young and adult gerbils. These studies have clearly shown that the same ischemic insult affect differently the synaptosomal uptake and release of 5-HT in all examined brain structures (except for the cortical 5-HT uptake, of the young and adult brain). Moreover, the observed regional differences in synaptosomal uptake and release in the brain of both young and adult animals suggest that they may represent one of the factors responsible for selective vulnerability of ischemic injury.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 NS 02275-10 LNNS</b>
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Prostaglandin Receptors in Cultured Glia and Cerebromicrovascular Smooth Muscle*</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
<b>PI:</b>	<b>M. Spatz</b>	<b>Section Chief</b>  <b>LNNS, NINCDS</b>
<b>Others:</b>	<b>B. Wroblewska</b> <b>O. Kempksi</b>	<b>Visiting Fellow</b> <b>Visiting Fellow</b>  <b>LNNS, NINCDS</b> <b>LNNS, NINCDS</b>
COOPERATING UNITS (if any)		
LAB/BRANCH <b>Laboratory of Neuropathology and Neuroanatomical Sciences</b>		
SECTION <b>Section on Neurocytobiology</b>		
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, MD 20892</b>		
TOTAL MAN-YEARS <div style="text-align: center;">1.0</div>	PROFESSIONAL: <div style="text-align: center;">.2</div>	OTHER: <div style="text-align: center;">.8</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             The effect of prostaglandins (PGE<sub>1</sub>, PGE<sub>2</sub>, PGF<sub>1α</sub>, PGF<sub>2α</sub>, PGD<sub>2</sub>) on adenylate cyclase (AC) was investigated in cerebromicrovascular smooth muscle and glial cell cultures. PGE<sub>1</sub> and PGE<sub>2</sub>, in contrast to PGF<sub>1</sub> and PGF<sub>2</sub>, stimulated cAMP formation in both cell types. The smooth muscle AC system showed a greater affinity for E-type prostaglandins than the AC system in glial cells. Most importantly, a dose-dependent PGI<sub>2</sub>, but not PGD<sub>2</sub> enhancement of cAMP formation, was manifested in smooth muscle while a reverse AC reactivity of PGI<sub>2</sub> and PGD<sub>2</sub> was seen in glial cells. These findings indicate that the prostaglandin receptors linked to AC are distinct in each cell type.           </p> <p>*[Formerly "Cerebral Capillary Endothelial Cultures: Prostaglandin Synthesis"]</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02357-08 LNNS

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Effects of Ischemia on Synaptosomal Serotonin Uptake and Release\*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Spatz	Section Chief	LNNS, NINCDS
Others:	K. Kumami	Visiting Fellow	LNNS, NINCDS
	V. Cvejic	Visiting Fellow	LNNS, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

.8

PROFESSIONAL:

.1

OTHER

.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Serotonin (5-HT) is both a central neurotransmitter and vasoactive agent. This monoamine exerts a wide range of metabolic and vascular effects on the cerebral circulation so it may well be involved in the mechanisms that link neuronal activity to cerebral blood flow.

Recently, we had demonstrated that the changes in the cerebral cortical content of 5-HT, its precursor tryptophan and its main metabolite 5-hydroxyindole acetic acid, unlike those of energy metabolites, depend on the age of the animal and the duration of ischemia. To shed some more light on this phenomenon, we studied the effect of ischemia on synaptosomal uptake and release of 5-HT in cerebral cortex, hippocampus and striatum of young and adult gerbils. The findings indicated that the same ischemic insult affected differently the synaptosomal uptake and release of 5-HT in all examined brain structures (except for the cortical 5-HT uptake) of the young and adult brain. Moreover, the observed regional differences in synaptosomal uptake and release in the brain of both young and adult animals may represent one of the factors responsible for selective vulnerability of ischemic injury.

\*[Formerly "Therapeutic GHB Effect on Experimental Cerebral Ischemia in Mongolian Gerbils"]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02429-07 LNNS
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Coordinate changes in brain energy metabolism and protein synthesis *		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) T. S. Nowak, Jr., Senior Staff Fellow, LNNS, NINCDS		
COOPERATING UNITS (if any) M. J. Schlesinger, Washington University of St. Louis Brian Cox, USUHS, Bethesda		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 1.0	PROFESSIONAL 1.0	OTHER 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided ) <p>Changes in brain energy metabolism and protein synthesis have been studied following <u>transient bilateral ischemia in the gerbil</u>. Project areas include: a) continued characterization of the stress or heat shock response following transient ischemia; b) evaluation of mechanisms responsible for the ischemia-induced <u>depletion of hippocampal dynorphin</u>; and c) mechanisms of overall <u>protein synthesis inhibition</u> and recovery following ischemia.</p> <p>The initial demonstration of increased translation of the major mammalian stress protein, hsp 70, is being extended to the transcriptional level. Preliminary results demonstrate the induction of mRNA's for hsp 70 and ubiquitin, using rat cDNA probes, following hyperthermia in the gerbil, and the effects of ischemia can now be investigated. Immunohistochemical localization of hsp 70 is being pursued with monoclonal antibodies specific for the induced protein.</p> <p>An earlier observation of dynorphin A depletion in gerbil brain following ischemia has been localized exclusively to hippocampus. The time course of changes in peptide levels is consistent with increased activity of the <u>dynorphin-containing dentate granule cells</u> following ischemia. These results extend the concept of hippocampal vulnerability to the neurochemical level, and suggest a role for the intrinsic hippocampal circuitry in proposed excitotoxic mechanisms for loss of hippocampal CA1 neurons following ischemia.</p> <p>*[Formerly in LNC]</p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02548-05 LNNS

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of electrical impedance in cerebral ischemia

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

H.G. Wagner, Chief, Section on Cerebrovascular Pathophysiology, LNNS, NINCDS

K. Kito, Visiting Fellow, LNNS, NINCDS

M. Seida, Visiting Fellow, LNNS, NINCDS

I. Klatzo, Chief, LNNS, NINCDS

S. Xu, Visiting Fellow, LNNS, NINCDS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section on Cerebrovascular Pathophysiology

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.2

## PROFESSIONAL:

0.9

## OTHER:

.3

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A series of cats were subjected to MCAO for periods 20 minutes up to 3 hours and followed for up to 6 hours post occlusion. In addition to measuring changes in the CEI, and the rCBF, the water content of selected sites was determined at various times up to 6 hours post occlusion. The permeability of the blood-brain barrier was also assessed by Nafluorescein tracer. Particular attention was given to acquiring a careful and accurate set of data on 20 minutes of occlusion of the MCA with the rCBF at or below 12 ml/100gm/min. Release of the occlusion was followed by immediate and marked hyperemia, significantly higher in the caudate than in the cortex (ectosylvian gyros) even though the rCBF's were the same. The hyperemias correlated well with the permeability of the BBB to NaFl. The deleterious role of the marked hyperemias was evident also in greater edema in the caudate. Morphological study also revealed a correlation with evidence of neuron damage which tended to disappear in about 2 weeks.

Longer duration ischemias, (more than 1 hr) showed greater edema but the impedance continued to return close to the preischemic values. Longer duration ischemias revealed gross and less recovery of the impedance to the preocclusion values.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02622-03 LNNS
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Cell Volume in Endothelial and Smooth Muscle Cell*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. Spatz	Section Chief LNNS, NINCDS
Others:	O. Kempinski	Visiting Fellow LNNS, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Neurocytobiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS 1.2	PROFESSIONAL .1	OTHER 1.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)		
<p>Our recent studies demonstrated that cerebromicrovascular endothelial cells exposed to hypotonic environment <u>in vitro</u> have a full capacity to normalize their volume and membrane potential but a limited capacity to restore the intracellular pH. The present study represents a continuous effort to elucidate the microvascular cellular properties and mechanisms involved in ensuring homeostatically controlled environment in the brain.</p> <p>The response of viable smooth muscle cells to medium of half normal osmolality was similar to that observed in endothelium. It was manifested by immediate cellular swelling without evidence of changes in the permeability to macromolecules or rapid recovery with complete normalization of cell volume.</p> <p>Both cell types (endothelium and smooth muscle) released the following amino acids: taurine, glutamate, aspartate and threonine, apart from K<sup>+</sup> ions during normalization of cellular volume. These results strongly suggest that the cerebrovascular endothelium and muscle cells have a built-in capacity for self-regulation which undoubtedly is important for normal function of blood-brain barrier (BBB).</p> <p>*[Formerly "Effects of Hypoosmotic Solutions on Cultured Cerebrovascular Endothelium"]</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 NS 02623-03 LNNS

PERIOD COVERED  
October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ischemic Brain Edema: Effect of  $\gamma$ -hydroxybutyrate on  $S_2$ -Receptor Binding Sites\*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Spatz	Section Chief	LNNS, NINCDS
Others:	V. Cvejic	Visiting Fellow	LNNS, NINCDS
	K. Kumami	Visiting Fellow	LNNS, NINCDS
	B. Wroblewska	Visiting Fellow	LNNS, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH  
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION  
Section on Neurocytobiology

INSTITUTE AND LOCATION  
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 2.0	OTHER: .5
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CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our previous results demonstrated that the ischemic edema is associated with an increased release of 5-HT and kinetic changes in the properties of synaptosomal serotonin ( $S_2$ )-receptor binding sites. In another study we have also shown that either preischemic or postischemic treatment of gerbils with  $\gamma$ -hydroxybutyrate (GHB), an endogenous central nervous system depressant, reduces the formation of edema and stabilizes the 5-HT metabolism. To shed some more light on the pathomechanisms of edema formation, we investigated the preischemic GHB effect on  $S_2$ -receptor binding sites in the ischemic model of edema on gerbils subjected to 15 min bilateral carotid artery occlusion and release for 1 hr.

These studies have shown that GHB given prior to the induction of bilateral carotid artery occlusion in gerbils prevented the ischemically induced changes in  $S_2$ -receptor binding sites using  $^3H$ -ketanserin as ligand (which labels specifically  $S_2$ -receptor sites). Thus, the capability of GHB to reduce the formation of ischemic edema and stabilize the 5-HT metabolism (observed in previous studies), as well as to prevent ischemic changes in the kinetic properties of  $S_2$ -receptor binding sites, reinforces the contention of GHB effect of 5-HT metabolic pathway.

\*[Formerly "Serotonin( $S_2$ )-Receptors in Ischemic Brain Edema"]

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02627-03 LNNS
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Relationship between electrical impedance and intracranial pressure</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <b>H.G. Wagner, Chief, Section on Cerebrovascular Pathophysiology, LNNS, NINCDS</b> <b>M. Seida, Visiting Fellow, LNNS, NINCDS</b> <b>K. Kito, Visiting Fellow, LNNS, NINCDS</b> <b>I. Klatzo, Chief, LNNS, NINCDS</b>		
COOPERATING UNITS (if any)  None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>AN EVALUATION OF THE ROLE OF INCREASED INTRACRANIAL PRESSURE (ICP) IN PRODUCING <u>BRAIN COMPRESSION</u> AND CHANGES IN <u>CEREBRAL ELECTRICAL IMPEDANCE</u> (CEI)</p> <p>Our studies showed that focal brain ischemia produced by three hour occlusion of the middle cerebral artery in cats produced a rise in the cerebral electrical impedance of the affected grey matter. The CEI returned approximately to pre-ischemic levels when the occlusion was released. In most of these animals a second rise in CEI was observed to occur later which appeared to be related to an increase in intracranial pressure. To test this hypothesis, brain compression was produced by epidural balloon inflation. When the epidural pressure was increased, the CEI increased as much as 216%. The regional blood flow (rCBF) was lowered but not necessarily to ischemic levels. This finding indicated that brain compression produced by edema can itself produce a reduction in extracellular space without always lowering rCBF to critical ischemic levels.</p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02689-02 LNNS

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Regulation of Carbohydrate Metabolism in Cerebromicrovascular Cultures\*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Spatz Section Chief LNNS, NINCDS

Others: H. Yamamoto Visiting Fellow LNNS, NINCDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section on Neurocytobiology

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS

1.2

## PROFESSIONAL

.1

## OTHER

1.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The regulation of norepinephrine induced glycogenolysis has been investigated in cultured cerebromicrovascular cellular elements. Preliminary studies revealed that the glycogenolytic effect of norepinephrine can be partially prevented by  $\beta_2$ -adrenergic blockers in endothelial cultures.

\*[Formerly "Effects of Vasoactive Substances in Carbohydrate Metabolisms in Cultured Elements"]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 NS 02690-02 LNNS</b>
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Forskolin Effects on Glial and Cerebromicrovascular Cultures*</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	M. Spatz	Section Chief LNNS, NINCDS
Others:	O. Kempfski	Visiting Fellow LNNS, NINCDS
	B. Wroblewska	Visiting Fellow LNNS, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH <b>Laboratory of Neuropathology and Neuroanatomical Sciences</b>		
SECTION <b>Section on Neurocytobiology</b>		
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, MD 20892</b>		
TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.3	.8	.5
CHECK APPROPRIATE BOXES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>The role of cyclic nucleotides in growth control and differentiation is yet unresolved. The recent availability of forskolin, a drug which directly stimulates the catalytic subunit of adenylate cyclase, allowed to study growth regulation of brain cells by cAMP from a new viewpoint. Glial cells, endothelium and smooth muscle cells from brain microvessels were used for this <u>in-vitro</u> study. cAMP production, thymidine incorporation, and morphological and cytoskeletal changes were examined.</p> <p>The results demonstrated that in all three cell types, thymidine incorporation was reduced dose-dependently with maximal growth inhibition at 100 <math>\mu</math>M forskolin. A one hour preincubation with forskolin abolished thymidine incorporation in fetal calf serum (FCS)-containing medium over the following 24 hours. Exposure to forskolin led also to drastic and immediate changes of cell morphology and F-actin composition in endothelium and smooth muscle. These changes were reversible. In glial cells morphologic changes were visible only after exposure to forskolin for more than 24 hours. These changes were accompanied by increased staining with antibodies against glial fibrillary acidic protein.</p> <p>The findings support a role of cAMP in growth regulation of these cells and indicate that forskolin might be used as a tool to induce growth arrest and eventually differentiation in cell cultures from mammalian brain.</p> <p>*[Formerly "Effects of Forskolin on the Growth and Differentiation of Cultured Cells from Rat Brain"]</p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02692-02 LNNS

## PERIOD COVERED

October 1, 1985 to September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphological evaluation of glycogen changes in cerebral ischemia

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

D.E. von Lubitz, Visiting Associate, LNNS, NINCDS

H. Masaoka, Visiting Fellow, LNNS, NINCDS

G. Goping, Biological Laboratory Technician, LNNS, NINCDS

I. Klatzo, Chief, LNNS, NINCDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

## SECTION

Section on Cerebrovascular Pathology

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.4

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The morphological changes in histochemically demonstrable glycogen were evaluated following 5 minute bilateral carotid occlusion in gerbils. The first appearance of abnormal increase in glycogen granules was observed in hippocampus after 2 hours following 5 minute ischemia. The accumulation of glycogen in astrocytic cells reached its peak at 6 hours after release of carotid occlusion. This was followed by a striking reduction in glycogen, especially in hippocampus, which was observed at 24 hours. A maximal accumulation of glycogen was conspicuous in Schaffer's collaterals at 48 hr post-ischemic time interval. These observations indicate that periods of previously demonstrated neuronal hyperactivity are associated with a conspicuous reduction of glycogen, whereas a collapse of neuronal activity corresponds to a conspicuous accumulation of glycogen, mainly in astrocytic cells. The morphological observations on glycogen provide thus an insight into changes in energy metabolism in cerebral ischemia and they contribute to a better understanding of this so clinically and important condition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 NS 02694-01 LNNS</b>
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Catecholamine Degradating Enzymes in Cerebromicrovascular Cultures</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; padding: 5px 0;"> <span><b>PI: M. Spatz</b></span> <span><b>Section Chief</b></span> <span><b>LNNS, NINCDS</b></span> </div>		
COOPERATING UNITS (if any) <b>C.R. Creveling, Laboratory of Bioorganic Chemistry, NIAMDD; I. Nagatsu, Fujita-Gakuen University School of Medicine, Toyoka, Japan; T. Nagatsu, Nagoya School of Medicine, Nagoya, Japan</b>		
LAB/BRANCH <b>Laboratory of Neuropathology and Neuroanatomical Sciences</b>		
SECTION <b>Section on Neurocytobiology</b>		
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, MD 20892</b>		
TOTAL MAN-YEARS: <div style="text-align: center;"><b>1.0</b></div>	PROFESSIONAL: <div style="text-align: center;"><b>.8</b></div>	OTHER: <div style="text-align: center;"><b>.2</b></div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The activity of catechol-O-methyltransferase (COMT) was investigated in cultured and propagated cerebromicrovascular endothelial and smooth muscle cells using high performance liquid chromatography and immunocytochemistry. The existence of COMT was detected in both cell types. The demonstration of this enzyme activity in the cerebromicrovascular smooth muscle cells, in addition to the endothelium, indicates that the enzymatic barrier to catecholamine is not limited to capillaries, the main constituents of the blood-brain barrier.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02702-01 LNNS

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathophysiological aspects of

blood-brain barrier (BBB) permeability in epileptic seizures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C. Nitsch, Visiting Scientist, LNNS, NINCDS

G. Goping, Biologist Laboratory Technician, LNNS, NINCDS

I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Blood-brain barrier (BBB) permeability to macromolecules was assessed during seizures induced by pentylentetrazole, bicuculline, methoxyppyridoxine, methionine sulfoximine, and kainic acid. It was observed that each convulsant induced a specific pattern of regional BBB opening. This was, however, only the case when systemic blood pressure (BP) rose with seizure onset. The analysis of regional cerebral blood flow revealed that a high increase in flow in rabbits with BP rise is related to the normal flow at rest in the single brain region, but not to BBB permeability. In rabbits without BP increase, regional flow increase was low but well modulated and is possibly a better indicator for neuronal activity. The ultrastructural analysis showed that macromolecular transport over the cerebrovascular endothelium is by pinocytosis, a neurotransmitter controlled process. It is suggested that seizure-induced regional BBB opening is determined by two factors: release of neurotransmitters due to the process of autoregulation during peripheral pressure increase, and change in local neurotransmitter milieu due to the action of the convulsant and/or the seizure activity.

The project is completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02703-01 LNNS
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Influence of blood-brain barrier opening to proteins on development of post-ischemic brain injury</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  P. Ting, Special Expert, LNNS, NINCDS H. Masaoka, Visiting Fellow, LNNS, NINCDS T. Kuroiwa, Visiting Fellow, LNNS, NINCDS H. G. Wagner, Chief, Section of Cerebrovascular Physiology, LNNS, NINCDS I. Fenton, Biologist, Laboratory Technician, LNNS, NINCDS I. Klatzo, Chief, LNNS, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.2	PROFESSIONAL 0.1	OTHER 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided)  <p>The effect of the BBB opening to proteins on development of post-ischemic brain injury was assessed in 32 cats subjected to one hour MCA occlusion. The CBF was measured by hydrogen clearance from electrodes inserted in the caudate and the cerebral cortex within the MCA territory. In 16 animals, a prevention of subsequent reactive hyperemia was attempted by hypovolemia, produced by withdrawal of blood just before the release of MCA occlusion. The hypovolemia was successful in prevention of post-ischemic hyperemia in five out of eight cats sacrificed at 3 hr and in six out of eight animals killed after 3 and 14 days. In cats sacrificed 3 hr after release of MCA occlusion, ischemic sites, associated with reactive hyperemia, showed evidence of BBB breakdown to proteins and significantly more severe edema than at the ischemic sites without reactive hyperemia, which otherwise failed to reveal leakage of EB tracer. In the cats sacrificed at 3 and 14 days, the ischemic sites which showed reactive hyperemia after release of MCA occlusion, revealed much more severe ischemic brain tissue injury than was observed at the sites without reactive hyperemia, which also did not show any EB leakage. In animals, which during the MCA occlusion showed similar intensity of ischemia in the caudate and the cerebral cortex, revealed usually more pronounced hyperemia in the former. The post-ischemic edema and the ischemic tissue injury appeared to be generally more severe in the caudate than in the cerebral cortex.</p> <p>The present studies indicate that reactive hyperaemia, which follows release of major cerebral artery occlusion, may play a significant role in the breakdown of the BBB to proteins, and in increasing the severity of post-ischemic edema and of ischemic brain tissue injury.</p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02704-01 LNNS
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on transient ischemic attacks (TIAs) in the experiment gerbil model		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) S. Tomida, Visiting Fellow, LNNS, NINCDS T. Nowak, Staff Fellow, LNNS, NINCDS K. Vass, Visiting Associate, LNNS, NINCDS J. Lohr, Biological Laboratory Technician, LNNS, NINCDS I. Klatzo, Chief, LNNS, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.8	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  The experimental model for TIAs, developed in gerbils, represents the first model which can be used in an easy, reproducible way to study systematically various aspects concerning the effect of repeated ischemic attacks upon brain tissue. The preliminary observations using this model indicate that there is a cumulative effect of repeated ischemic insults, when they are carried out at time intervals at which there is post-ischemic hypoperfusion. The cumulative effect of repeated ischemic insults is expressed in intensity of edema and brain tissue injury, which in 3 repeated insults is considerably higher than resulting from a single ischemic insult of duration equal to sum of individual repetitive attacks.		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02718-01 LNNS
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Study of cerebral electrical activity associated with ischemia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) H.G. Wagner, Chief, Section on Cerebrovascular Pathophysiology, LNNS, NINCDS S. Xu, Visiting Fellow, LNNS, NINCDS		
COOPERATING UNITS (if any)  None		
LAB/BRANCH Laboratory of Neuropathology and Neuroanatomical Sciences		
SECTION Section on Cerebrovascular Pathophysiology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">1.1</div>	PROFESSIONAL: <div style="text-align: center;">1.0</div>	OTHER: <div style="text-align: center;">0.1</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  A study of electrical activity, how it is affected by ischemia and edema, and how recovery of activity is related to temporary ischemic insults of graded durations. During this period, instrumentation has been procured and or constructed, optimized and familiarized. Preliminary experiments have begun.		







# ANNUAL REPORT

October 1, 1985 through September 30, 1986  
Laboratory of Neurophysiology

National Institute of Neurological and  
Communicative Disorders and Stroke

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Laboratory of Neurophysiology, IRP  
National Institute of Neurological and  
Communicative Disorders and Stroke  
Jeffery L. Barker, M.D., Chief

During FY 86 the research program emerging in the Laboratory of Neurophysiology continued to develop following the period of expansion in FY 85. All of the projects involve elucidation of specific molecular or cellular properties either under in vitro or in situ conditions. Although the spectrum of research activity already established or in development is quite diverse, all of the experiments remain contemporary in technique and strategy, if not innovative. The combination of quantitative structural and functional assays into important cell biological properties common to many cellular phenotypes, especially the developmental and molecular biology of specific transmitters and receptors, is considerable. The collective expertise and experience now in the Laboratory has the potential to realize some of the long-term goals of our overall research program.

One goal involves quantitative analysis of the ontogenetic development and phylogenetic distribution of specific transmitter receptors. When do certain receptors develop during evolution or arise during development, by what synthetic mechanisms, and how are receptors distributed in functionally distinct nerve, endocrine, immune and cardiac muscle tissues? What are the molecular characteristics of the receptors when studied from the level of the genome to transduction of transmitted-mediated signals initiated in the membrane? What is the anatomic disposition of specific receptor proteins in membranes, and what is their topographic distribution along the surface of specific cellular phenotypes? Another related goal involves quantitative analysis of specific receptor functions in phenotypically distinct nerve, endocrine and immune cells using contemporary cell biological assay techniques. How are specific receptors related to ion conductance mechanisms in the membrane or to regulation of cytosolic pCa or to other receptor-coupled membrane and cytoplasmic properties? By focussing on several major classes of transmitters and their receptors we should be able to discover fundamental aspects of receptor structure and function and slowly but surely gain some insight into a "transmitter code", especially as it applies to specific circuits of nerve, endocrine and immune cells.

It has become indelibly clear that transmitters and receptors and receptor-regulated changes in membrane excitability are virtually ubiquitous, being expressed to a variable extent in all cells and in some organelles studied thus far. Most of our present projects involve characterization of specific receptor properties and functions exhibited by cellular elements derived from specific regions of the embryonic and adult vertebrate CNS, from clonal and primary endocrine pituitary tissues, from heart and from human and rodent clonal and primary immune tissues. There are a variety of experimental strategies underway, some of which aim to hold the question constant, while varying the membrane, and some of which hold the membrane constant and vary the question. These include: 1) high-yield receptor protein purification for adrenergic and cholinergic receptors; 2) analysis of the primary aminoacid sequence and structure of adrenergic and cholinergic receptors; 3) synthesis of different domains of receptor protein followed by immunization in vivo and in vitro and the production of monoclonal anti-domain immunoreagents useful in functional assays; 4) molecular biological studies into the genomic sequences

that encode for specific transmitter receptor proteins in functionally distinct tissues and systems; 5) dissociated primary and clonal cultures of mammalian nerve, endocrine and immune tissues; 6) quantitative electrophysiological and optical recording techniques applied at the single-cell and monosynaptic circuit levels in dissociated cell cultures, in retinal and hippocampal "slice" and retinal eyecup preparations; 7) flow cytometric analysis of physiologically important properties in cellular suspensions of embryonic CNS tissue, clonal and primary pituitary tissue and immune cells; 8) flow cytometric isolation of specific cellular phenotypes from nerve, endocrine and immune systems; 9) light and electron-microscopic resolution of cellular form and subcellular structure in monolayer culture preparations and in normally developed spinal cord, sensory ganglia, hippocampus and retina; 10) immunohistochemical characterization of transmitter phenotype and surface-receptor expressions in sorted and unsorted monolayers of embryonic CNS cells and normally organized retinal tissue; and 11) quantitative analysis of fluorescence signals expressed by cytoplasmic and membrane determinants in cultured nerve, endocrine and immune cells.

This array of biotechnology is considerable and diverse, yet complementary. With this range of experience and expertise we should be able to discover when specific receptors become expressed both during embryogenesis and in the course of evolution and what roles they play in the physiological context of chemical signalling between functionally distinct phenotypes. The strength of the Laboratory lies in the collection of innovative strategies and the opportunity for interdependent research. We now have the resources to ask questions regarding the structure and function of specific transmitters and receptors in experimental detail, examining their roles in intercellular communications between specific nerve, muscle, endocrine and immune cells.

The implementation by Drs. Venter, Fraser, Kerlavage and Chung of innovative receptor-protein-purification protocols, coupled with receptor protein sequence analysis and, more recently, molecular biological probing of genomic sequences encoding receptor protein has certain promise. Elucidation of receptor structure and the production of receptor-specific reagents to study receptor function in physiological and pathophysiological conditions are worthwhile and important lines of interdependent investigation to pursue. Equally significant has been the development of relatively routine flow cytometric analysis of cell suspensions followed by isolation and monolayer culture of embryonic mammalian motoneurons, surface-labelled mesencephalic cells, primary prolactinergic and growth hormone pituitary cells and specific effector lymphocytes by Drs. Schaffner, DiPorzio, St. John, Mandler, Ms. Novotny and Ms. Smallwood. For example, we should soon be able to detect and analyze with flow-cytometry specific receptor and ion-channel distributions in populations of nerve, endocrine and immune cells and then isolate and culture these cells for detailed multi-disciplinary study of receptor function at the single-cell level using the various quantitative electrical, optical and morphological techniques in the Laboratory. Ideally, the dual-color capability of the cell-sorter will reveal not only receptor expression in subpopulations of phenotypically distinct cells but also simultaneously record certain receptor-coupled functions probed with indicator dyes.

In summary, the Laboratory has developed a strong and varied research program to study the biology of specific receptors expressed in a variety of cellular phenotypes and their roles in physiologically important circuits involving nerve, muscle, endocrine and immune cells. Eventually, we plan to compare data obtained on specific receptor biologies expressed in normal tissues and systems with that found either in certain pathophysiological states or in spontaneous or "gene-engineered" mutants to uncover the possible receptor-related mechanisms involved and identify innovative therapies.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02019-14 LNP

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Electrophysiological Studies on Membrane Excitability

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.L. Barker, Chief, LNP, IRP, NINCDS. Others (LNP, IRP, NINCDS) T.G. Smith, Section Chief; G.D. Lange, Physiologist; M.K. Walton, Senior Staff Fellow; P.A. Sheehy, Staff Fellow; G.-G.Chen, Fogarty Fellow; N.L. Harrison, Fogarty Fellow; S.V.P. Jones, Fogarty Fellow; J.W. Harrington, Physiologist; R.W. Turner, Guest Researcher; V. Smallwood, Bio. Lab. Technician

## COOPERATING UNITS (if any)

D.E.R. Myers (LNLC, NINCDS); M.A. Rogawski (MNB, NINCDS); K. Inoue (FDA); L. Skirboll (CNB, NIMH); M.D. Majewska (CNB, NIMH); C. Evans (LB, NCI); S. Barnett (LB, NCI); D. Greenblatt (LMI, NIAID); H.A. Wilson (LMI, NIAID).

## LAB/BRANCH

Laboratory of Neurophysiology, IRP, NINCDS

## SECTION

Office of the Chief and Section on Sensory Physiology

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

12

## PROFESSIONAL:

11

## OTHER:

1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this research program involves elucidation of the ion permeability mechanisms expressed by primary cells cultured from the embryonic mammalian CNS, from endocrine pituitary and from immune tissues. These mechanisms are considered critical in the physiology and diverse functions of the various cellular phenotypes. Specific lines of investigation include projects on embryonic CNS neurons cultured from spinal and supraspinal regions, clonal and primary pituitary cells, and clonal and primary effector lymphocytes as well as their tumor targets. Electrophysiological measurements of excitability are made in membrane patches or in whole cells, using either low-resistance patch-clamp pipettes or high-resistance microelectrodes for recording. The different assay techniques provide complementary data for characterizing the membrane and cytoplasmic mechanisms underlying ion conductances in these cells. Principal observations this year include the following: 1) simultaneous whole-cell patch-type electrical recordings from cultured GABAergic and target hippocampal neurons in functional synaptic contact; 2) quantitative analysis of GABA-mediated synaptic signals; 3) structure-activity-study of requirements for steroid modulation of GABA receptor-coupled Cl<sup>-</sup> conductance: active steroids occur naturally as metabolites of certain hormones and may amplify inhibitory signals throughout the CNS; 4) discovery of a relatively novel depolarizing conductance that may be proximal to epileptogenic activity; 5) transient-type K<sup>+</sup> channels in clonal pituitary cells have the same elementary conductance as delayed-type K<sup>+</sup> channels but distinct pharmacologies; 6) prolactin and growth-hormone cells sorted from the adult rat pituitary express excitable membrane properties in culture; 7) interleukin-2 induces delayed-type anionic conductance mechanisms in putative killer-type lymphocytes; 8) leukoregulin triggers the transient appearance of depolarizing ion channel activity.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02330-09 LNP
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Cell Biological Studies of CNS Neurons, Pituitary and Immune Cells</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J.L. Barker, Chief, LNP, IRP, NINCDS. Others (LNP, IRP, NINCDS): D. Cosenza-Murphy, Chemist; U. DiPorzio, Visiting Scientist; G.D. Lange, Physiologist; R.N. Mandler, Visiting Associate; E. A. Novotny, Biologist; P.A. St. John, Senior Staff Fellow; A.E. Schaffner, Senior Staff Fellow; V. Smallwood, Bio. Lab. Technician		
COOPERATING UNITS (if any) G. Rougon (LCB, NIMH); M. Fordis (LMB, NCI); B. Howard (LMB, NCI); P. Henkart (CI, NCI); P. Kushner (ALS Fdn. San Francisco); M. Constantine-Paton (Yale Univ.); R. Barchi (U. of Penn.); R. Drugan (CNB, NIMH); R. Weber (CNB, NIMH)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 10.0	PROFESSIONAL: 6.5	OTHER: 3.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The immediate and continuing aim of this line of investigation involves the development of strategies to probe the development and differentiation of <u>phenotypic properties</u> expressed by <u>functionally distinct types of nerve, endocrine and immune cells</u>. The strategies in development include <u>dual-laser fluorescence-activated cell analysis and sorting (FACS)</u>, <u>light-microscopic characterization of cells in monolayer culture</u> using <u>immunoreagents</u> reacting with <u>cytoplasmic and surface antigens</u> and <u>semi-quantitative analysis of optically detectable fluorescence signals</u> emanating from cells in monolayer culture. Principal observations this year include: 1) implementation of <u>voltage-sensitive indicator dye technology</u> for use with the FACS, application to cell suspensions of embryonic rat spinal cord, initial characterization of a <u>complex distribution of membrane potentials</u> arising in vital elements, and discovery of functional <u>Na<sup>+</sup> channel expression</u> developing earlier on spinal elements than sensory cells; 2) implementation of <u>surface-immunoreactions</u> for quantitative analysis of antigen expression in nerve, endocrine, immune and renal cells on the FACS; 3) routine isolation of nerve and endocrine cells based on surface-immunoreaction signals in the FACS; 4) days-long culture of adult pituitary <u>prolactin and growth hormone cells</u>; 5) implementation of <u>Ca<sup>2+</sup>-indicator dye technology</u> to monitor intracellular <u>Ca<sup>2+</sup> levels</u> in single clonal pituitary cells with initial observations on spontaneously arising fluctuations in <u>Ca<sup>2+</sup> levels</u> and hormone-induced changes in <u>Ca<sup>2+</sup> levels</u>; 6) <u>cell-cycle analysis</u> on the FACS of cells <u>co-transfected with senescent DNA and the gene encoding for interleukin-2</u>; and 7) preliminary data showing that natural killer-type lymphocyte cytotoxicity and mitogen-stimulated lymphocyte <u>proliferation</u> are <u>compromised</u> in a behavioral model of <u>fear-conditioning</u>.           </p>		

## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01659-18 LNP

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synaptic Contacts of Retinal Neurons

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Lasansky, Chief, Section on Cell Biology, LNP, IRP, NINCDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Neurophysiology, IRP, NINCDS

## SECTION

Section on Cell Biology

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1

## PROFESSIONAL:

1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neuronal surfaces in retinal slices are occasionally clean enough to allow for Giga-ohm seals with blunt electrodes. Initial attempts have yielded recordings in the whole-cell mode from depolarizing bipolar cells.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01-NS-02631-03 LNP
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function in Retinal Neurons		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: R. Nelson, Physiologist, LNP, IRP, NINCDS. Others (LNP, IRP, NINCDS): A.P. Mariani, Special Expert; M. Freed, Guest Researcher; R. Pflug, Visiting Associate; J.N.M. deMelo, Fogarty International Fellow. H. Kolb, Professor Physiol. Dept., U. of Utah; J.I. Korenbrot, Professor, Physiol. Dept., U. of California; G.J. Chader, Chief, LVR, NEI; E.K. Barbehenn, Pharmacologist, DMEDP, FDA		
COOPERATING UNITS (if any) Department of Physiology, University of Utah, Salt Lake City (H. Kolb); Department of Metabolism and Endocrine Drug Products, FDA (E.K. Barbehenn); Laboratory of Vision Research, NEI (G. Chader).		
LAB/BRANCH Laboratory of Neurophysiology, IRP, NINCDS		
SECTION Neural Circuitry Unit		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In the <u>outer plexiform layer</u> of the <u>cat retina</u> large field background stimuli are able to enhance the responses of <u>horizontal cells</u> to small red flickering spots. The responses to the spots originate with the red cones; the enhancement effect of the background originates mainly with rods, as demonstrated by spectral sensitivity studies. These results demonstrate a new <u>rod-cone interaction</u> and suggest a different role for the horizontal cell 'feedback' <u>synapse onto cones</u>, at least for these rapid stimuli. In the dark, when horizontal cells are depolarized, an inhibitory transmitter is released onto the cones, reducing the amplitude of response. The light-induced hyperpolarization of horizontal cells releases cones from this inhibition. The mechanism stands in contrast to classic horizontal cell feedback, where horizontal cell hyperpolarization reduces the cone response, but after some delay.</p> <p>The <u>dopaminergic amacrine cells</u> of <u>monkey retina</u> have been studied using <u>immunocytochemical methods</u>. Using an <u>antibody</u> directed against <u>tyrosine hydroxylase</u> as a marker, these cells have been seen to receive input from <u>bipolar cells</u>, as well as other amacrine cells, as previously reported. The bipolar appears to be a specific type called 'giant bistratified' and is connected specifically to cones.</p> <p>At the inner margin of the retina, a second type of glial cell, the <u>fibrous astrocyte</u>, has been found to respond electrically to natural <u>photic stimulation</u>. These cells, which are imbedded in the optic nerve fiber layer, respond with transient depolarizations to the onset and cessation of light stimuli. Their responses probably reflect the release of <u>potassium from ganglion cell axons</u> passing through the nerve fiber layer. Previously, <u>Muller cells</u> were the only retinal <u>glial cells</u> known to have light responses.</p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02705-01 LNP

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anatomical Studies of Neurons, Neurotransmitters and Neurotransmitter Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A.P. Mariani, Special Expert, LNP, IRP, NINCDS. Others (LNP, IRP, NINCDS): J.L. Barker, Chief; J.N. deMelo, Fogarty International Fellow; D. Cosenza-Murphy, Chemist; M. Lee, Student Volunteer

## COOPERATING UNITS (if any)

J.C. Venter, Chief, Section of Receptor Biochemistry, LNP; H. Mohler, Hoffman-La Roche, Basle, Switzerland

## LAB/BRANCH

Laboratory of Neurophysiology

## SECTION

Office of The Chief

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland, 20892

## TOTAL MAN-YEARS:

2.45

## PROFESSIONAL:

2.2

## OTHER:

0.25

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

The component elements of the neuronal circuitry, their synaptic organization, neurotransmitters and neurotransmitter receptors are studied with electron microscopy and light and electron microscopic immunohistochemistry. Monoclonal antibodies against purified benzodiazepine/GABA Cl<sup>-</sup> channel complex (BZR) have shown that BZR is expressed on the surface of rat spinal and hippocampal neurons grown in tissue culture. In the primate retina, BZR and glutamate decarboxylase (GAD) immunoreactivities were largely non-overlapping showing that BZR is not exclusively associated with GABAergic synapses. Thus the immunohistochemistry provides evidence for 2 major classes of GABAergic synapses, those which bind benzodiazepines and those which do not, as well as benzodiazepine binding in the absence of GABAergic synaptic transmission.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02670-02 LNP
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Evolution of Neurotransmitter Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J.C. Venter, Section Chief, LNP, IRP, NINCDS. Others (LNP, IRP, NINCDS): C.M. Fraser, Research Physiologist; S. Fracek, Staff Fellow; S.M. Shreeve, Fogarty Associate; A. Kerlavage, Senior Staff Fellow; D. Robinson, Biologist; J. Earle, Biologist; J. Lai, Fogarty Fellow; K.-U. Lentes, Guest Researcher; J. Gocayne, Biologist; P.C. Potter, Fogarty Fellow		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurophysiology, IRP, NINCDS		
SECTION Section on Receptor Biochemistry		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.8	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The purpose of this project is to examine the <u>structural and functional evolution of neurotransmitter receptor proteins</u>. Using <u>muscarinic cholinergic and beta adrenergic receptors</u> as the initial model systems we have isolated and characterized these receptors from the <u>brains and hearts</u> of a wide variety of species. Brain and heart tissue have been obtained from all vertebrate classes including <u>mammals, birds, reptiles, amphibians, and fish</u> as well as from lower species including <u>insects (Drosophila)</u>. Data from muscarinic receptor studies demonstrate that out of all of the parameters studied (SDS gel molecular weight, isoelectric point, monoclonal antibody cross reactivity, agonist affinity, antagonist affinity, stereospecificity of ligand binding, GTP shifts of agonist affinity, and receptor density) only receptor density appears to have changed over <u>900 million years of evolution</u> gradually increasing in higher species. In contrast, beta receptor structure has appeared to change more dramatically over the past 400 million years.</p> <p><u>Muscarinic receptors</u> are being <u>purified from human brain, rat brain and heart, pig heart and shark brain and heart</u> in order to compare <u>primary structures</u> in detail. The evolutionary relationship between muscarinic cholinergic receptors and other neurotransmitter receptors is also being studied. Protein sequence data and defined gene probes will be employed to follow the evolution of <u>adrenergic, cholinergic and GABAergic receptors</u>.</p> <p>Neurotransmitter <u>biosynthetic/degradative enzymes</u> have been examined using receptor-specific monoclonal antibodies. In addition, <u>primary sequence</u> data for these proteins has been compared with that of neurotransmitter receptor proteins for any evolutionary or structural relationships. Gene cloning of adrenergic and cholinergic receptors is underway from a wide variety of species to study specific receptor domains involved in such functions as ligand binding and coupling to other membrane effectors.</p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02671-02 LNP

## PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Immunological Characterization and Localization of Neurotransmitter Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C.M. Fraser, Research Physiologist, LNP, IRP, NINCDS. Others (LNP, IRP, NINCDS): J.C. Venter, Section Chief; S.M. Shreeve, Fogarty Associate; J. Earle, Biologist; D. Robinson, Biologist; D. Cosenza-Murphy, Biologist; J. Gocayne, Biologist; P.C. Potter, Fogarty Fellow

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Neurophysiology, IRP, NINCDS

## SECTION

Section on Receptor Biochemistry

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

3.0

## PROFESSIONAL:

2.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aims of this research are to develop extensive libraries of polyclonal and monoclonal antibodies to major classes of neurotransmitter receptors including muscarinic cholinergic, alpha and beta-adrenergic, GABAergic and opiates; and to utilize these reagents in studies of receptor structure and function, the degree of molecular homology between receptor subclasses, the localization of neurotransmitter receptors on cell membranes, the evolution of receptor subclasses and the role of anti-receptor antibodies in human diseases.

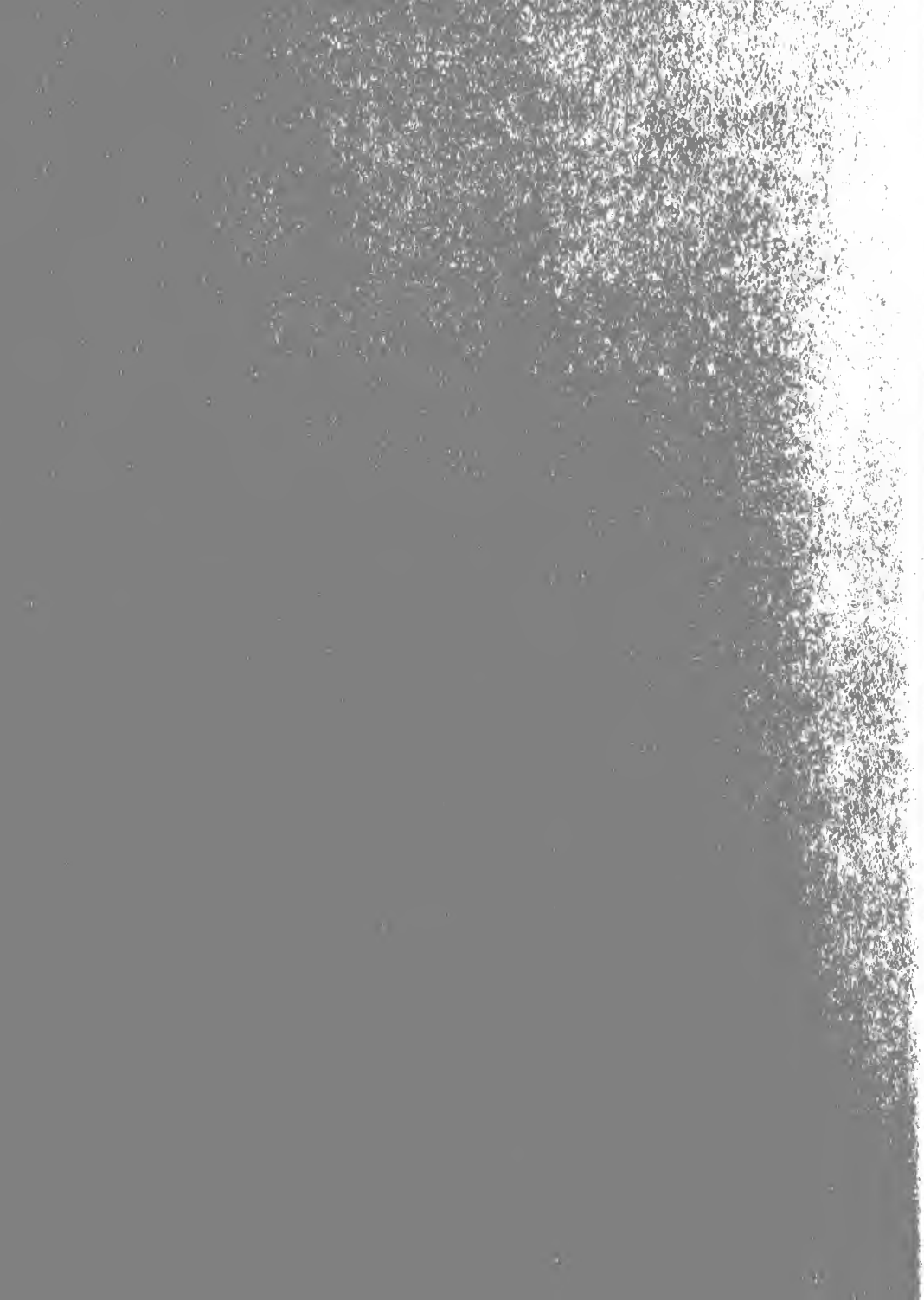
Principal observations to date include the following: 1) monoclonal antibodies raised against each major class of neurotransmitter receptor recognize the same receptor in a number of different tissues and species suggesting considerable conservation of receptor structure throughout evolution; 2) certain monoclonal antibodies raised against muscarinic cholinergic receptors recognize alpha<sub>1</sub>-adrenergic receptors and vice versa suggesting structural homology between these pharmacologically distinct receptor sub-types; 3) monoclonal antibodies raised against alpha<sub>1</sub>-adrenergic receptors recognize alpha<sub>2</sub>-adrenergic receptors, and this information taken together with pharmacological and biochemical data suggests that alpha<sub>1</sub>- and alpha<sub>2</sub>-adrenergic receptors may be closely related "isoreceptors"; 4) monoclonal antibodies to muscarinic cholinergic receptors have been utilized to fluorescently label receptors on the surface of cultured cells and in brain sections demonstrating the potential usefulness of these reagents in studies of receptor localization and distribution in various species and tissues as well as in studies of receptor processing and turnover; and 5) some patients suffering from depression possess autoantibodies to human brain membrane proteins. The identity of these proteins is being pursued.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02672-02 LNP
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Neurotransmitter Receptor Purification and Structure</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J.C. Venter, Section Chief, LNP, IRP, NINCDS. Others (LNP, IRP, NINCDS): C.M. Fraser, Research Physiologist; S.M. Shreeve, Fogarty Associate; S. Fracek, Staff Fellow; A. Kerlavage, Senior Staff Fellow; D. Robinson, Biologist; J. Earle, Biologist; J. Lai, Fogarty Fellow; K.-U. Lentz, Guest Researcher; A. Mariani, Senior Staff Fellow; J. Gocayne, Biologist; P.C. Potter, Fogarty Fellow		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurophysiology, IRP, NINCDS		
SECTION Section on Receptor Biochemistry		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 4.8	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Neurotransmitter receptors; adrenergic (beta<sub>1</sub>, beta<sub>2</sub>, alpha<sub>1</sub> and alpha<sub>2</sub>), cholinergic (muscarinic and nicotinic), and benzodiazepine receptors are being isolated and purified in order to understand the molecular basis of receptor function and neuronal communication. Specific projects are underway to provide precise structural information on each of the above receptor proteins. <u>Structural data</u> being obtained include <u>primary sequence data</u>, <u>proteolytic digest maps</u>, <u>topology</u> information and structure-function data, e.g., neurotransmitter binding site localization, sugar localization, membrane domain and effector coupling protein recognition domains. The structure of receptors from Alzheimer's and Huntington's disease patients are being examined by immunological techniques.</u> </p> <p> <u>Our data have demonstrated that structural similarities exist among non-pharmacologically related neurotransmitter receptors (muscarinic, cholinergic and alpha adrenergic) and that these neurotransmitter receptors mediate cellular modulation via protein conformational changes initiated by neurotransmitter binding to the binding site in the extracellular protein domain. <u>Receptor coupling</u> is mediated by the cytoplasmic "tail" of the receptors which appears to be the effector protein (GTP-regulatory protein) recognition portion of the receptor. Electron microscopy, performance size-exclusion chromatography of purified receptors indicate that the alpha<sub>2</sub> and beta<sub>2</sub> receptors exist as homodimers while the muscarinic receptor is monomeric.</u> </p> <p> <u>Protein preparative procedures have been established which include various HPLC steps, ligand affinity chromatography, monoclonal antibody affinity chromatography, preparative SDS-gel electrophoresis, lectin affinity chromatography, ion exchange chromatography and column isoelectric focusing. The establishment of these purification protocols is now permitting simultaneous detailed structural comparisons of all adrenergic and cholinergic receptor proteins.</u> </p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02710-01 LNP
PERIOD COVERED <u>October 1, 1985 to September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Molecular Biology of Neurotransmitter Receptors</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  PI: J.C. Venter, Section Chief, LNP, IRP, NINCDS. Others (LNP, IRP, NINCDS): F.-Z. Chung, Staff Fellow; C.M. Fraser, Research Physiologist; K.-U. Lentz, Guest Researcher; J. Lai, Fogarty Fellow; D. Robinson, Biologist; S. Fracek, Staff Fellow; J. Gocayne, Biologist; P.C. Potter, Fogarty Fellow; A. Kerlavage, Senior Staff Fellow		
COOPERATING UNITS (if any)  None		
LAB/BRANCH <u>Laboratory of Neurophysiology, IRP, NINCDS</u>		
SECTION <u>Section of Receptor Biochemistry</u>		
INSTITUTE AND LOCATION <u>NINCDS, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS: <div style="text-align: center;">3.0</div>	PROFESSIONAL: <div style="text-align: center;">2.8</div>	OTHER: <div style="text-align: center;">0.2</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           We are in the process of <u>cloning the genes</u> for major classes of <u>neurotransmitter receptors</u> including <u>cholinergic</u>, <u>adrenergic</u> and <u>GABAergic</u>. <u>cDNA libraries</u> have been obtained or are being constructed from <u>human brain</u>, <u>heart</u> and <u>lung</u>, and <u>porcine</u>, <u>shark</u>, and <u>Drosophila</u> tissue for <u>evolutionary comparison</u> of receptor structures to test the hypothesis developed by this group that neurotransmitter receptors are part of a <u>multigene family</u>.         </p> <p>           Full length genes are being utilized as probes for <u>mRNA synthesis</u> in developmental models for receptors.         </p> <p>           To date we have isolated two human brain cDNA clones which cross hybridized to oligonucleotide probes specific for the N-terminal, middle and C-terminal regions of the beta2-adrenergic receptor gene. The nucleotide sequences of these two cDNA clones are being determined by the M13-dideoxy chain termination method.         </p> <p>           In order to determine the number of copies of beta-adrenergic receptor present in the genome, genomic DNAs from human, porcine, shark, etc., will be examined by <u>Southern blot analysis</u>. <u>Genomic DNA libraries</u> from various species will be constructed to screen for these potentially different genes. The cloned genes will be expressed in different prokaryotic and eucaryotic expression vectors to study the molecular structure of these receptors. <u>Site-directed mutagenesis</u> will be used to study the <u>structure-function relationship</u> of these receptors.         </p>		









ANNUAL REPORT  
October 1, 1985 through September 30, 1986

Biometry and Field Studies Branch

Intramural Research Program  
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT  
for period October 1, 1985 through September 30, 1986

Biometry and Field Studies Branch

Intramural Research Program

National Institute of Neurological and Communicative Disorders and Stroke

Jonas H. Ellenberg, Ph.D., Chief

The Biometry and Field Studies Branch (BFSB) supports a program in biostatistics to advance the mission of NINCDS in the areas of neurologic and communicative disorders. The Branch participates in a wide range of intramural and extramural collaborative projects, including large- and small-scale observational studies, clinical trials and laboratory studies. These collaborative studies are conducted both through direct staff research and through research and development contracts. In addition to collaborative work, the Branch has an important research component in statistical methodology.

The Branch has an Office of the Chief and three Sections. The Mathematical Statistics Section, headed by Dr. James Dambrosia is the main statistical consulting group for other branches and laboratories in the Intramural Research Program as well as the Extramural Research Program. The Section also is responsible for research in statistical methodology. The Computer Applications Section is mainly occupied with activities related to the Stroke and Traumatic Coma Data Banks. The Branch Chief is Acting Chief of this Section, although Dr. Mary Foulkes is Project Director for both of the Data Banks. The Data Processing Section provides computer programming, systems analysis and data management support to the other Sections and other Programs in the Institute and is headed by Ms. Sylvia Edelstein. The Surveys and Demographic Studies Section has been abolished. Dr. James Dambrosia has been named Acting Deputy Chief of the Branch to assist the Branch Chief in policy decisions and the administrative burden of running the Branch.

Issues of staffing, the mix and nature of our collaborative efforts and the time available for methodological research have been under extensive evaluation over the past year. Many of the projects we currently support are long-term and labor intensive, and involve a heavy commitment of staff. These projects require an enormous amount of our staff time to be spent on "operations" type activities such as data entry, data editing and day to day monitoring of protocol compliance. Although we have initiated changes which will relieve us of some of this type of work load, we will have to wait several years for normal close-out of many of these projects before any real relief is evident. This type of activity, although essential, consumes such a large amount of our staff time that little time is left for statistical methodology and expansion of our collaborative efforts within the Institute. BFSB collaboration on new projects with a large data management component will be carefully considered, and our participation will be contingent on the routine daily data management operations of such studies being contracted out, under our supervision. We have included in our budget for FY '86, direct operations monies to fund a data management and programming contract which will provide data entry, data monitoring, computer programming and systems analysis in partial support of ongoing and new collaborative projects.

We have recruited two doctoral level statisticians, both with a combination of strong theoretical training and excellent consulting backgrounds. One of the statisticians is located administratively in the Mathematical Statistics Section, and she will work in methodology and in collaboration with other Institute scientists. The second new senior staff member is assigned to the Office of the Chief and has assumed responsibility as Project Director of our Stroke and Traumatic Coma Data Banks. Her extensive experience in the statistical and administrative operations of many multicentered collaborative studies has proven of considerable importance in establishing rational and clear cut directions for successful completion of these projects. Although the new staff members will have heavy collaborative workloads, we expect that their presence will allow BFSB staff additional time to focus on studies of statistical methodology in collaborative research.

#### I. COLLABORATION WITH THE INTRAMURAL AND EXTRAMURAL RESEARCH PROGRAMS, NINCDS

Our current collaborative research program has developed primarily in response to requests for collaboration from Intramural and Extramural scientists at NINCDS. Typically, BFSB assumes responsibility for the statistical design, data management, statistical analysis and interpretive aspects of the projects, with the Program providing the project initiatives, subject matter expertise and overall project leadership. In addition the Branch is engaged in research in statistics as it applies to problems encountered in clinical, laboratory, and epidemiological studies.

In collaboration with the Convulsive, Developmental and Neuromuscular Disorders Program (CDNDP), BFSB is the statistical coordinating center for the clinical trial of behavioral and cognitive side effects of phenobarbital used for the prevention of febrile seizure recurrence. Patient accrual has been completed, with 217 children with febrile seizures randomized to treatment and 150 seizure free controls recruited for the study. Follow-up of patients will continue until June, 1988. BFSB has been the comprehensive operations center for this study, which has required extensive monitoring of patient accrual and data quality control and several interim data analyses for the trial's monitoring committee. The study had made use of the Medical Studies Data Base System developed by BFSB staff using an in-house HP-1000 computer, but system hardware and software problems dictated a change in data management systems. At great expense of staff time and effort the entire data base management system had to be rewritten for the NIH computer and all existing HP-1000 data transferred to the DCRT/IBM computer. The new system is now operational.

A second collaborative effort with the CDNDP is a population-based study of the prognostic value of the EEG for subsequent seizure activity in children who experienced a febrile seizure. The cooperating medical center is the Pediatric Clinic in Skopje, Yugoslavia. The recruitment of new cases ended in December, 1984, and periodic follow-up (including repeat EEG's and neurologic and physical examinations) will continue through December, 1987. The study includes 400 children with a normal or non-specific abnormal EEG following a first febrile seizure, as well as about 200 children with a specific abnormal EEG following a seizure. The major outcomes of the study are recurrent febrile and afebrile seizures and their relationship to the initial EEG, subsequent EEG changes, and the influence of other medical and demographic factors.

BFSB with CDNDP has completed the survey of medical practice in the management of children with febrile seizures. This survey was based on a probability sample of approximately 10,000 physicians on the American Medical Association list of child neurologists, pediatricians, family practitioners and general practitioners. The primary information obtained from the survey concerned method of treatment, factors that determine treatment or consultation, reasons for chronic treatment, and preferred medications. A manuscript reporting the major medical findings has been accepted for publication in the American Journal of Diseases of Children and another report analyzing the characteristics of the physicians who responded to the survey is in preparation.

BFSB is participating with the Communicative Disorders Program on a study of factors associated with the acquisition of reading and writing skills by the deaf. Information on educational and family background and on language skills will be examined to determine which, if any, of these variables are associated with reading and writing skills in deaf adolescents. Planning and study design have been completed, data collection forms have been tested, and accrual of subjects is now in progress.

Six interventional therapy studies for the treatment of acute stroke are in various stages of completion. These studies are conducted under the Cerebrovascular Clinical Research Master Agreement of the Stroke and Trauma Program. The primary role of BFSB in these studies is that of data management. Patient accrual and follow-up have been completed on three studies: a dose escalation study of Naloxone, a Phase II Naloxone study based on the results of the dose escalation study, and a dose escalation/pilot study of hypervolemic hemodilution (Dextran-40). These three studies used the HP-1000 Data management system, which encountered system and software problems prior to completion of the studies. The computer problems could not be resolved and the data from the three studies have been transferred to the DCRT/IBM computer. Other studies being conducted under the Master Agreement include: the use of Nicardipine, a calcium channel blocker, for the prevention of vasospasm following subarachnoid hemorrhage; the treatment of acute cerebral ischemia with Nicardipine and the treatment of acute cerebral ischemia with heparinoid. These studies are in progress and use the IBM/PC for forms design, data collection, data management, and report generation. Patient accrual and follow-up for these latter three studies will be completed in FY '87.

The BFSB continues to collaborate with many Branches and Laboratories in the Intramural Research Program (IRP). The Survey of Major Neurological Disorders in Copiah County, a joint project with the Neuroepidemiology Branch, is completed. Reports on stroke and epilepsy have been published this year. A paper summarizing all of the findings of the survey has been accepted for publication. A report on functional disability has been submitted for publication. The successful completion of this project, with its important findings with regard to racial differences for major neurologic disorders, is a major joint accomplishment of the two Branches. The methodology developed for this project provides an effective model for other studies attempting to obtain prevalence rates of neurologic disorders in geographically defined populations.

BFSB is working with the Medical Neurology Branch on a clinical trial of Felbamate, a new drug for the treatment of intractable partial seizures. The

planning and statistical design of the study have been completed and the study is now underway. The study uses a randomized, double-blind, three-period-crossover design, allowing unbiased estimation of treatment effect even in the presence of period-to-period carry-over effect. The duration of the study will be approximately two and one-half years with each patient hospitalized for about three months.

Other collaborative studies in IRP include: an evaluation of cyclohexyl adenosine treatment of stroke in a gerbil model (LNNS and LN); a characterization of vocal tremor and acoustic discrimination of normal control from cases with throat disorders (NM); statistical aspects of the epidemiology of Creutzfeld-Jakob disease with emphasis on clustering (LCNSS); comparison of the relationship between the number of myelin lamellae and axon size in normal and "shiverer" strains of mice (LENP); a pilot study of von Recklinghausen's neurofibromatosis (NE); analysis of time to stroke using time dependent covariates in a proportional hazards regression model (NE); a clinical trial of ganglioside therapy for diabetic neuropathy (MN); a case-control study of stroke in hospitalized persons to compare risk factors between whites and blacks (NE); a study of the potential association of toxoplasmosis during pregnancy with morbidity in the child (ID); and analysis and comparison of the amplitude of blink responses evoked by mechanical or electrical stimuli (MN).

The major analyses of data from the Collaborative Perinatal Project (NCPPI) in collaboration with CDNDP has been completed. Work in the primary areas of cerebral palsy and convulsive disorders was finished at the end of the 1985 calendar year. A paper on the effects of seizures on intellectual capacity as measured by IQ and one on the multivariate analysis of the antecedents of cerebral palsy were both published in the New England Journal of Medicine. Another paper on the multivariate analysis of the antecedents of seizures in young children has been accepted for publication in the American Journal of Diseases of Children. Work on the maternal infection studies from this data base is nearing completion. A paper on the relationship of toxoplasmosis during pregnancy with childhood outcome has been accepted for publication in the Journal of Infectious Diseases. A study investigating the association of migraine with other diseases has been completed and will be submitted for publication, and another assessing the familial relationship of migraine in mothers and morbidity in their children is in progress.

## II. CLINICAL DATA BANKS

BFSB continues its responsibility for the funding, management and operation of the Stroke and Traumatic Coma Data Banks. Each data bank is a collaborative effort between BFSB, which is the statistical coordinating center, and four hospital centers. The data banks involve the collection of prospective, observational, clinical and laboratory data at the several clinical centers using a common set of data forms. These data banks will provide a data resource for addressing research questions on the characteristics, clinical course, and outcome of hospitalized stroke and coma patients.

Data collection, processing and editing involve the coordinated efforts of BFSB, a contractor for systems analysis and computer programming support (RLR and



Associates), and the clinical data bank centers. BFSB has designed and implemented a data base system at NIH (DCRT) and a patient tracking system to monitor patient accrual and completed follow-up visits. RLR and Associates' workscope has been expanded from design, maintenance, and telecommunications aspects of the "front-end" micro-computer system to include transfer of data from the micro-computer to DCRT, and programming support for the building and updating of the databases at DCRT. DCRT is the data repository for each of the data banks and data are now transmitted directly to DCRT via the micro-computer "front-end" system.

For the duration of both data banks, BFSB will continue to emphasize monitoring of data collection to enhance consistency of procedures at the collaborating centers, to require prompt entry of collected data, and to increase the rate of successful follow-up. Resources will be allocated to ensure a satisfactory database when these studies are completed. Any major efforts at analysis, publication of preliminary results or any other uses of resources which put achievement of this objective at risk will be avoided.

### Stroke Data Bank

Data collection for the main phase of the Stroke Data Bank began in FY '83. By the end of new patient accrual in June, 1986, over 1,700 patients will have been entered. All acute care data will be entered by the centers, edited and corrected by BFSB and a final acute care data file created by the Fall of 1986. Primary analysis of acute care data will begin at that time. Collection of follow-up data will continue until March, 1987, and the project will end in June, 1987.

Examples of research studies to be addressed using the final edited data base include: the identification of patients at risk for evolving ischemic stroke, defined as a change in the Glasgow Coma Scale, a change in weakness, or the development of new neurologic deficits; the investigation of the hypothesis of racial and sexual differences in stroke type, site and vascular territory; and the examination of stroke severity to determine whether motor weakness and/or sensory loss can be predicted by the location or size of the CT scan abnormality in infarcts. A logistic regression model based on the Pilot Stroke Data Bank patient records has been developed to identify early during hospitalization a group of patients unlikely to survive for one month following their intracerebral hemorrhage. This model is now being evaluated for its predictive ability in the main phase of the Stroke Data Bank.

### Traumatic Coma Data Bank

Data collection for the main phase Traumatic Coma Data Bank began in FY '84. By the end of May, 1986, over 600 severely head-injured patients will have been enrolled in the study.

Monitoring of CT scan readings has led to improvements in both the coding of CT scans and in the definitions employed within the Traumatic Coma Data Bank. Differences across centers in ICP data capture, diagnostic workups, aggressive-ness of follow-up and in patient demographics are each being assessed for their effects on the evaluation of major hypotheses and the potential generalization of results.

The assessment of the ability of surviving patients to function may represent an important outcome measure in the Traumatic Coma Data Bank. The long-term impact of severe head injury on life quality is profound, but the exact nature of this impact, and its evolution over time is still poorly documented. The Katz Adjustment Scale and the Sickness Impact Profile, coupled with clinician ratings of mental functioning will provide data that can be utilized to better understand the long-term impact of severe head injury on the patient's life. Increased emphasis has been placed on consistent, unbiased and complete follow-up of surviving coma patients to permit the detailed evaluation of outcome.

### III. METHODOLOGICAL RESEARCH IN STATISTICS

BFSB statisticians continue to develop new statistical methodology and derive innovative modifications of statistical techniques to meet the needs of the Institute for the design of experiments and field studies, analysis of data, and statistical modeling of biological processes and phenomena. Most of the statistical problems addressed arise from collaborative studies with the Intramural and the Extramural Programs. In general, there are two objectives associated with these various statistical activities of BFSB. The primary objective is the development and improvement of statistical methodology to meet the needs of the Institute. The secondary objective is to make contributions to the development of statistical methodology which may be more generally useful in neurologic and other medical research.

A partial listing of areas in which BFSB staff is developing new statistical applications to neurologic problems includes: modified tests for space-time clustering of rare disease applied to a population in a defined geographic area; an autoregressive model of patient response for a k-period-2-treatment crossover drug trial that accounts for both treatment residual effects and random effects for the individual patient; use of incomplete observations in statistical models derived by stepwise variable selection procedures; methods for adjustment of the effect of concomitant variables in categorical data analysis; sampling strategies for rare neurologic disorders; and the application of proportional hazards regression with time dependent risk factors to the analysis of stroke etiology.

Theoretical statistical work has included: the effect of misclassification of exposure variables on case-control studies; the non-null distribution of statistics that measure spatial clustering; new hypothesis testing procedures in the presence of inequality constraints; nonparametric methods for quadratic alternatives; the effect of informative censoring on survival analysis; and examination of the biases introduced by left-truncation of time-dependent covariates in the analysis of proportional hazards.

### IV. OUTSIDE ACTIVITIES

Several of our staff have been active on important national and international review committees or invited to participate in major meetings this year. Dr. Ellenberg was invited as a member of a small UNICEF sponsored group of international scientists to review the work of the Peoples Republic of China in

the area of child development, and to lecture at various Chinese medical centers. He also accepted an invitation by the Institute of Medical Risk Studies to help define the future research objectives and priorities for the utilization of California data bases for deriving information on the prevention of cerebral palsy. Dr. Anderson has been invited to present two plenary lectures at the Pan American Congress of Neuroepidemiology in Santiago, Chile on the design and quality control of epidemiological surveys. Dr. Raubertas co-authored a presentation "Hypothesis Tests for Normal Means Constrained by Linear Inequalities," an invited presentation at the Conference on Order-Restricted Inference sponsored by the University of Iowa. Dr. Foulkes is a member of the monitoring committee for the VA Cooperative Study of Carbamazepine versus Divalproex for treatment of partial seizures.

In summary, BFSB is involved in a strong program of collaborative research. Our collaboration extends throughout the Institute on projects with both Intramural and Extramural scientists, and also involves collaboration with scientists outside of NINCDS. The broad scope of our research activity ranges from small, one-on-one collaboration with intramural scientists, to the conduct of large-scale, multicenter clinical data banks. BFSB also makes an important and continuing contribution to statistical methodology applicable to neurological research. We have strengthened our position and emphasis in this area as indicated through our recruitment and allocation of resources.

CONTRACT NARRATIVE  
Biometry and Field Studies Branch, IRP, NINCDs  
Fiscal Year 1986

1. UNIV. OF MARYLAND (N01-NS-2-2302)
2. NEUROLOGICAL INSTITUTE - COLUMBIA UNIV. (N01-NS-5-2384)
3. BOSTON UNIV. (N01-NS-2-2398)
4. MICHAEL REESE HOSPITAL & MEDICAL CENTER (N01-NS-2-2399)

Title: Full Phase Stroke Data Bank

Date Contracts Initiated: July 1, 1982

Contractors' Principal Investigators:

1. Dr. Thomas Price
2. Dr. Jay Mohr
3. Dr. Philip Wolf
4. Dr. Daniel Hier

Current Annual Levels FY '86:

1. \$257,000
2. \$356,000
3. \$209,000
4. \$252,000

Objectives: The primary objective of this project is to implement the full phase of the Stroke Data Bank study, which will collect observational acute and long-term longitudinal data on stroke patients. The data bank will provide a resource for clinical research studies of patients with stroke. This is a collaborative project which involves four clinical centers and BFSB. The clinical centers are responsible for the collection of data and collaboration on the development of and analysis of research questions. The BFSB, is the statistical coordinating center, responsible for maintenance of the centralized data base management system for transmission, for storage and retrieval of data; for monitoring of data acquisition and data quality; and for collaboration with the clinical investigators on the analysis of data concerning the primary research questions of the data banks.

Methods Employed: The Steering Committee, composed of the Principal Investigators and BFSB personnel, met during the first year of this project, outlined research objectives, and developed forms and data collection procedures. The focus this fiscal year has been on the improvement of data entry procedures, the improvement of follow-up rates, the elimination of data entry backlog, the creation of edited data files for analysis, and the creation of analysis teams for the primary research questions. In addition, a monitoring committee was convened by the Branch to provide continuing critical review of the Stroke Data Bank to both the Branch Chief and the IRP Director.

Significance to the NINCDS Program and Biomedical Research: Acute care and longitudinal data on stroke patients will be collected at four centers, using uniform definitions and procedures. This information will provide a large body of data for clinical research on the factors influencing survival and quality of life following a stroke.

Proposed Course of the Project: This is the beginning of the fourth year of a five-year project. Work in the first year included determination of research questions to be investigated and design of forms to collect the data. Data collection began in July, 1983, and as of May, 1986, information on over 1,700 patients had been collected.

Publications:

Gross, C.R., Shinar, D., Mohr, J.P., Hier, D.B., Caplan, L.R., Price, T.R., Wolf, P.A., Kase, C.S., Fishman, I.G., Calingo, S. and Kunitz, S.C.: Inter-observer agreement in clinical studies: Application to the diagnosis of stroke type. Arch. Neurol. (In press)

Shinar, D., Gross, C.R., Price, T.R., Banko, M., Boldue, P.L. and Robinson, R.: Screening for depression in stroke patients: The reliability and validity of the center for epidemiologic studies depression scale. Stroke 17(2): 241-245, 1986.

Mohr, J.P., Rubinstein, L.V., Edelstein, S.R., Gross, C.R., Heyman, A., Kase, C.S., Kunitz, S.C. and Price, T.R.: Approaches to pathophysiology of stroke through the NINCDS Data Bank. In: Plum, F. (Ed.) Cerebrovascular Disease, 14th Princeton Conference, New York, Raven Press, 1985.

Robinson R.K., Starr, L.B., Lipsey J.R., Pao, K. and Price, T.R.: A two year longitudinal study of post-stroke mood disorders: In-hospital prognostic factors associated with six month outcome. J. Nerv. Ment. Dis. 173: 221-226, 1985.

Mohr, J.P., Nichols, F.T. and Tatemichi, T.A.: Classification and diagnosis of stroke. International Angiology. 3: 431-439, 1984.

CONTRACT NARRATIVE  
Biometry and Field Studies Branch, IRP, NINCDS  
Fiscal Year 1986

1. UNIV. OF TEXAS-GALVESTON (N01-NS-3-2339)  
AND BAYLOR UNIV. MEDICAL COLLEGE
2. UNIV. OF CAL. IN SAN DIEGO (N01-NS-3-2340)
3. MEDICAL COLLEGE OF VIRGINIA (N01-NS-3-2341)
4. UNIV. OF VIRGINIA (N01-NS-3-2342)

Title: Full Phase Traumatic Coma Data Bank

Date Contracts Initiated:

1. April 15, 1983
2. April 15, 1983
3. June 1, 1983
4. July 1, 1983

Contractors' Principal Investigators:

1. Dr. Howard Eisenberg
2. Dr. Lawrence Marshall
3. Dr. Harry Young
4. Dr. John Jane

Current Annual Level FY '86:

1. \$265,000
2. \$272,000
3. \$230,000
4. \$220,000

Objectives: The primary objective of this project is to implement the full phase of the Traumatic Coma Data Bank study, which will collect observational acute and long-term longitudinal data on severely head injured patients. The data bank will provide a resource for clinical research studies of patients with head injury. This is a collaborative project which involves four clinical centers and BFSB. The clinical centers are responsible for the collection of data and collaboration on the development of and analysis of research questions. The BFSB is the statistical coordinating center, responsible for maintenance of the centralized data base management system for transmission, storage and retrieval of data; for monitoring of data acquisition and data quality; and for statistical collaboration or oversight of the statistical analysis for the primary research questions of the data bank.

Methods Employed: The Steering Committee, composed of the Principal Investigators and BFSB personnel, met during the initial year of this project, outlined the research objectives, and developed forms and data collection procedures. Data collection began in January, 1984. During this fiscal year, the focus has been on the improvement of follow-up rates, the assessment of center differences in data collection procedures and corrections where necessary, the general

(N01-NS-3-2339)  
(N01-NS-3-2340)  
(N01-NS-3-2341)  
(N01-NS-3-2342)

improvement of data entry procedures and the elimination of data entry backlog. In addition, a monitoring committee has been established by the Branch Chief to provide continuing critical review of the Traumatic Coma Data Bank to both the Branch Chief and IRP Director.

Significance to the NINCDS Program and Biomedical Research: Acute care and longitudinal data on head-injured victims will be collected at four centers, using uniform definitions and procedures. This information will provide a large body of data for clinical research on the factors influencing survival and quality of life following severe head injury. The number of therapies and monitoring devices commonly utilized during the acute phase of managing traumatic coma necessitates a highly organized data handling capacity, and the data bank will serve as an efficient mechanism for collecting, storing and retrieving this information as well as follow-up data.

Proposed Course of the Project: This is the third year of a five-year project. Data collection is continuing and as of June, 1986, over 600 patients have been entered into the project.

Publications:

Ruff, R., Evans, R. and Green, R.: Long-term Remediation of Head Injured Patients and Support Groups. In Blumenthal, J.A., and McKee, D.C. (Eds.): A Clinician's Source Book. Miami, Professional Resources Exchange, 1986.

CONTRACT NARRATIVE  
Biometry and Field Studies Branch, IRP, NINCDS  
Fiscal Year 1986

RLR & ASSOCIATES, INC., Fairfax, Virginia (N01-NS-2-2315)

Title: Front-end Microprocessor Support for Data Bank Projects

Date Contract Initiated: June 30, 1982

Contractor's Project Director: Robert L. Rush

Current Annual Level FY '86: \$44,000

Objectives: To provide the Stroke and Traumatic Coma Data Bank projects (N01-NS-2-2302, 2398-9, N01-NS-5-2384, N01-NS-3-2339-42) with a front-end software package for cost-effective interactive data entry, updating, editing and nighttime transmission to a host computer, to provide support to the Data Bank Maintenance Center (the Computer Applications Section, BFSB), to provide a data base management system for efficient storage, retrieval and management of the collected data, and to design and implement software additions, enhancements, and maintenance of the existing system. (Note that in FY '85, the workscope and funding for this contract was modified to include responsibility for the creation and management of a data base management system, in addition to the front-end system.)

Major Findings: The contractor continued to provide considerable and significant support for data entry, storage, and retrieval for both the Stroke and Traumatic Coma Data Banks.

Significance to the NINCDS Program and Biomedical Research: The front-end and data base management systems are integral parts of the Stroke and Traumatic Coma Data Bank Projects, which were established to collect and maintain medical data for clinical research. Data storage, retrieval and management of the collected clinical data is essential to fulfill the research objectives of the Data Bank Projects.

Proposed Course of the Project: This contract terminates in August, 1986. The function will continue through the issuance of a new contract.

Publications: None



CONTRACT NARRATIVE  
Biometry and Field Studies Branch, IRP, NINCDS  
Fiscal Year 1986

TO BE AWARDED

Title: Collection and Maintenance of Data for the Stroke Data Bank  
and Traumatic Coma Data Bank Projects

Date Contract Initiated: September 1, 1986

Contractor's Project Director: TBA

Current Annual Level FY '86: \$152,000 (Estimated)

Objectives: To provide and maintain front-end microprocessor support for the Stroke and Traumatic Coma Data Bank projects (N01-NS-2-2302, 2398-9, N01-NS-5-2384, N01-NS-3-2339-42), with facilities for interactive data entry, updating, editing and transmissions to the NIH DCRT main-frame computer. As well, to provide data maintenance, retrieval and management of the stored data. To maintain and refine software for data monitoring procedures.

Major Findings: This contract is expected to provide substantial support for data entry, storage and retrieval for both the Stroke and Traumatic Coma Data Bank projects.

Significance to the NINCDS Program and Biomedical Research: The ability to enter and check data on-site via front-end software is an integral part of the Stroke and Traumatic Coma Data Bank projects. This contract will also provide the support for data management, storage and retrieval, necessary to fulfill the research objectives of both projects.

Proposed Course of the Project: As of the date this report was compiled, this contract had not yet been awarded. It is anticipated that the contract will run from September 1, 1986 through December 1, 1988.

Publications: None

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02652-02 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Intramural Statistical Collaboration and Consultation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James M. Dambrosia, Ph.D. Chief, Mathematical  
Statistics Section BFSB, IRP, NINCDS

Others: Dallas Anderson, Ph.D. Mathematical Statistician BFSB, IRP, NINCDS  
Young Jack Lee, Ph.D. Mathematical Statistician BFSB, IRP, NINCDS  
Richard Raubertas, Ph.D. Mathematical Statistician BFSB, IRP, NINCDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

1.7

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to provide statistical collaboration and consultation for Laboratories and Branches within the Intramural Research Program (IRP). Particular consideration is given to statistical planning and design of experiments, statistical analysis of data, and statistical inference. Our collaboration has involved eight Laboratories/Branches, and the scope of the studies has ranged from the coordination and statistical management of a complex clinical trial to consultation on the appropriateness of the statistical analysis used for small laboratory experiments. Examples of studies with IRP include a randomized clinical trial of Felbamate, a new drug for the treatment of intractable partial seizures (MN); an evaluation of efficacy of the drug cyclohexyl adenosine for treatment of ischemic stroke in a gerbil model (LNNS and LN); statistical aspects of the epidemiology of Creutzfeldt-Jakob disease with emphasis on case clustering (LCNSS); characterization of vocal tremor and acoustic discrimination of normal controls and cases with throat disorders (MN); examination of the relationship between the number of myelin lamellae and axon size in normal and 'shiverer' strains of mice (LENP); development of proportional hazards models for time to stroke with time dependent covariates (NE); a clinical trial of ganglioside therapy for diabetic neuropathy (MN); and a pilot study of von Recklinghausen's neurofibromatosis (NE).

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02490-06 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research in Statistics

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James M. Dambrosia, Ph.D. Chief, Mathematical

Statistics Section

BFSB, IRP, NINCDS

Others: Young Jack Lee, Ph.D.

Mathematical Statistician BFSB, IRP, NINCDS

Dallas W. Anderson, Ph.D.

Mathematical Statistician BFSB, IRP, NINCDS

Jonas H. Ellenberg, Ph.D.

Chief BFSB, IRP, NINCDS

Mary A. Foulkes, Ph.D.

Senior Staff Fellow BFSB, IRP, NINCDS

Richard F. Raubertas, Ph.D. Mathematical Statistician BFSB, IRP, NINCDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

1.0

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project addresses statistical problems generated from collaboration with scientists in other program areas and general statistical problems of current interest. This project is a continuing activity of the Section on Mathematical Statistics. Papers have been submitted or published in FY'86 on the following statistical subjects: pooled adjacent violators; general test of trend for count data; hypothesis tests involving linear inequality constraints; sampling strategies for rare diseases; the analysis of Phase II clinical trials; the development of robust selection procedures based on vector ranks; the evaluation of sample size and power for analysis of survival with allowance for non-uniform patient entry, losses to follow-up, non-compliance and stratification; and stochastic curtailing for comparison of slopes in longitudinal studies. Other work in progress includes: statistics for the evaluation of space-time clustering of disease; modeling of residual treatment effect for k-period two-treatment crossover designs; adjustments for covariates in the analysis of categorical data; the influence of missing data on statistical models determined by variable selection procedures; the effects of misclassification of exposure variables on case-control studies; the use of area surveys in epidemiological research; the impact of risk and incidence of risk on selection of variables in multiple regression; the development of methods to improve coverage in surveys; the use of non-parametric tests for umbrella type alternative hypotheses; the impact of informative censoring on survival analysis with time dependent covariates; and the development of methods of survival analysis for dependent competing risks.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02444-07 BFSB																					
PERIOD COVERED October 1, 1985 through September 30, 1986																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Statistical Coordinating Center for the Phenobarbital Clinical Study*</b>																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Young Jack Lee, Ph.D.</td> <td style="width: 33%;">Mathematical Statistician</td> <td style="width: 33%;">BFSB, IRP, NINCDS</td> </tr> <tr> <td>Others: Jonas H. Ellenberg, Ph.D.</td> <td>Chief</td> <td>BFSB, IRP, NINCDS</td> </tr> <tr> <td>Karin B. Nelson, M.D.</td> <td>Chief, Cerebral Palsy and Other Motor Disorders Sec.</td> <td>DNB, CDNDP, NINCDS</td> </tr> <tr> <td>Deborah G. Hirtz, M.D.</td> <td>Pediatric Neurologist</td> <td>DNB, CDNDP, NINCDS</td> </tr> <tr> <td>Jack Panossian</td> <td>Programmer</td> <td>BFSB, IRP, NINCDS</td> </tr> <tr> <td>Sylvia Edelstein</td> <td>Chief, Data Processing Sec.</td> <td>BFSB, IRP, NINCDS</td> </tr> <tr> <td>Dolores Jones</td> <td>Computer Assistant</td> <td>BFSB, IRP, NINCDS</td> </tr> </table>			PI: Young Jack Lee, Ph.D.	Mathematical Statistician	BFSB, IRP, NINCDS	Others: Jonas H. Ellenberg, Ph.D.	Chief	BFSB, IRP, NINCDS	Karin B. Nelson, M.D.	Chief, Cerebral Palsy and Other Motor Disorders Sec.	DNB, CDNDP, NINCDS	Deborah G. Hirtz, M.D.	Pediatric Neurologist	DNB, CDNDP, NINCDS	Jack Panossian	Programmer	BFSB, IRP, NINCDS	Sylvia Edelstein	Chief, Data Processing Sec.	BFSB, IRP, NINCDS	Dolores Jones	Computer Assistant	BFSB, IRP, NINCDS
PI: Young Jack Lee, Ph.D.	Mathematical Statistician	BFSB, IRP, NINCDS																					
Others: Jonas H. Ellenberg, Ph.D.	Chief	BFSB, IRP, NINCDS																					
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Jack Panossian	Programmer	BFSB, IRP, NINCDS																					
Sylvia Edelstein	Chief, Data Processing Sec.	BFSB, IRP, NINCDS																					
Dolores Jones	Computer Assistant	BFSB, IRP, NINCDS																					
COOPERATING UNITS (if any) Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS; University of Washington																							
LAB/BRANCH Biometry and Field Studies Branch																							
SECTION Mathematical Statistics Section																							
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:																					
2.8	1.2	1.6																					
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The Biometry and Field Studies Branch is the Statistical Coordinating Center for the ongoing clinical trial of the <u>behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence</u>. The accrual of patients has been completed, with 367 patients on study. Follow-up with psychometric testing will continue for another two years.</p> <p>The original data management system which dealt with data entry, data editing and data retrieval was developed for an HP mini-computer at the Biometry and Field Studies Branch. During the year, the system met with both hardware and HP software problems that necessitated a transfer of the data management capabilities to a new system. The entire data management system was recreated, and is now housed and operated on the main frame computer at NIH.</p> <p>During this fiscal year, three interim analyses of the data were performed for evaluation by the Performance and Safety Monitoring Committee. In addition, extensive data editing is continuing, creation of structured files for analysis is near completion, the distribution of the outcome variables have been examined and strategies are being developed for final analysis.</p> <p>*[This study supports the DNB/CDNDP/NINCDS contract study entitled: "Behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence." The project officer is Dr. Karin B. Nelson, DNB, CDNDP, NINCDS, and the contractor of the study is the University of Washington.]</p>																							

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02598-04 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stroke Data Bank\*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mary A. Foulkes, Ph.D.	Sr. Staff Fellow	BFSB, IRP, NINCDS
Others:	Irene G. Fishman, M.S.	Statistician	BFSB, IRP, NINCDS
	Gary Kamer	Programmer	BFSB, IRP, NINCDS
	Margaret Meadows	Statistician Asst.	BFSB, IRP, NINCDS
	Selma C. Kunitz, Ph.D.	Chief, Computer	BFSB, IRP, NINCDS
		Applications Section	
	Christine Wolf	Programmer	BFSB, IRP, NINCDS

## COOPERATING UNITS (if any)

Departments of Neurology: Boston U. Medical Center, Michael Reese Hospital,  
Neurological Institute -Columbia University, and University of Maryland

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Computer Applications Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.7

## PROFESSIONAL:

1.2

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Stroke Data Bank is a prospective observational study collecting data on hospitalized newly diagnosed stroke patients, at four clinical centers. The four collaborating clinical centers are responsible for the collection of acute care and longitudinal follow-up information using common definitions and procedures, under contracts N01-NS-2-2302, 2398-9, N01-NS-5-2384. The research objectives for the project were formulated by a steering committee composed of the principal investigators from the clinical centers, other outside experts, and BFSB staff, with the concurrence of the BFSB Advisory Committee. The research objectives were the basis for determining the specific data to be collected, the format of the data collection forms and the data collection procedures. The general objective for the project is to provide a large and comprehensive body of data for clinical research on the factors influencing survival and quality of life following onset of a stroke.

The BFSB serves as the statistical coordinating center for the project, providing an on-site front-end data entry system with interactive feedback for data editing; the data base management system for transmission, storage and retrieval of data, for monitoring of data acquisition and its quality; and for statistical collaboration with the clinical investigators for the analysis of the primary research questions.

The project is in its third year of data collection and has entered over 1700 patients as of May, 1986. The first major analyses will begin after accrual of patients has been completed (June, 1986) and the acute care data has been entered and edited (Fall, 1986). The focus of the Branch during the data collection period will be on monitoring the project with regard to completeness and quality of data collection.

\*[Formerly entitled: Complications, Recurrence, and Outcome: Stroke Data Bank]

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 NS 02516-05 BFSB</b>
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Traumatic Coma Data Bank*</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Mary A. Foulkes, Ph.D.      Sr. Staff Fellow	BFSB, IRP, NINCDS
Others:	Irene G. Fishman, M.S.      Statistician	BFSB, IRP, NINCDS
	Gary Kamer      Programmer	BFSB, IRP, NINCDS
	Selma C. Kunitz, Ph.D.      Chief, Computer Applications Section	BFSB, IRP, NINCDS
	Christine Wolf      Programmer	BFSB, IRP, NINCDS
	Margaret Meadows      Statistician Asst.	BFSB, IRP, NINCDS
COOPERATING UNITS (if any)  Depts. Of Neurology: Medical College of Virginia, University of California - San Diego, University of Texas - Galveston, University of Virginia		
LAB/BRANCH <b>Biometry and Field Studies Branch</b>		
SECTION <b>Computer Applications Section</b>		
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, Maryland 20892</b>		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The <u>Traumatic Coma Data Bank</u> is a prospective observational study collecting data on <u>severely head injured</u> patients at four clinical centers. The four collaborating centers are responsible for the collection of acute care and longitudinal follow-up information using common definitions and procedures, under contracts N01-NS-3-2339-42. The research objectives for the project were formulated by a steering committee composed of the principal investigators from the clinical centers, other outside experts, and BFSB staff, with the concurrence of the BFSB Advisory Committee. The research objectives were the basis for determining the specific data to be collected, the format of the data collection forms and the data collection procedures. The general objective for the project is to provide a large and comprehensive body of data for clinical research on the factors influencing <u>survival</u> and <u>quality of life</u> following a severe head injury.</p> <p>The BFSB is the statistical coordinating center for the project, providing an on-site front-end data entry system with interactive feedback for data editing; the data base management system for transmission, storage and retrieval of data, for monitoring of data acquisition and its quality; and for statistical collaboration with the clinical investigators and statistical analysis oversight for the primary research questions of the data bank.</p> <p>The project is in its second year of data collection and has entered over 600 patients as of June, 1986. The first major analyses will begin after accrual of patients has been completed, and the acute care data has been entered and edited. The focus of the Branch during the data collection period will be on the monitoring of the project with regard to completeness and quality of data collection.</p> <p><b>*[Formerly entitled: Traumatic Coma: Epidemiological Characteristics]</b></p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02596-04 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Data Bank Maintenance Center

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Irene G. Fishman, M.S. Statistician BFSB, IRP, NINCDS

Others: Ella Maneely Programmer BFSB, IRP, NINCDS

Gary Kamer Programmer BFSB, IRP, NINCDS

Selma C. Kunitz, Ph.D. Chief, Computer Applications Section BFSB, IRP, NINCDS

## COOPERATING UNITS (if any)

RLR &amp; Associates, Inc., Fairfax, Virginia

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Computer Applications Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

0.2

## OTHER:

1.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Coordination of the data management activities for the Stroke and Traumatic Coma Data Banks (N01-NS-2-2302, 2398-9, N01-NS-5-2384, N01-NS-3-2339-42), as well as design of the data storage, data retrieval, and system enhancements are the responsibility of the Computer Applications Section (CAS), which is the statistical coordinating center for the data banks. Each data bank has four cooperating hospital centers collecting data prospectively on the acute and long-term follow-up of stroke and coma patients. The data are stored at NIH (DCRT) in SAS data sets, after they are transmitted from the clinical centers. The RLR and Associates' contract workscope (N01-NS-2-2315) provides for programming of the software for the host data base management system for DCRT and for the editing procedures and transmission of data from the centers to DCRT through a front-end system. RLR and Associates will maintain both the front-end aspects and the host data base of the system. Retrieval of data for analysis is a shared responsibility of the individual clinical center sites with the Computer Applications Section.

A patient tracking system was designed, developed and implemented for the Traumatic Coma and Stroke Data Banks. This system, in which data are entered directly by the clinical centers into DCRT, monitors the flow of patients, as well as forms completion, from entry into the study, through follow-up.

\* [This project has been subsumed under the projects: Traumatic Coma Data Bank (Z01-NS-02516-05) and Stroke Data Bank (Z01-NS-02598-04).]

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02595-04 BFSB
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Methodological Aspects of Data Banks*</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div> <b>PI: Irene G. Fishman, M.S. Statistician</b>   <b>Other: Selma C. Kunitz, Ph.D. Chief, Computer Applications Section</b> </div> <div style="text-align: right;"> <b>BFSB, IRP, NINCDS</b>   <b>BFSB, IRP, NINCDS</b> </div> </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH <b>Biometry and Field Studies Branch</b>		
SECTION <b>Computer Applications Section</b>		
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, Maryland 20892</b>		
TOTAL MAN-YEARS: <div style="text-align: center;">0.0</div>	PROFESSIONAL: <div style="text-align: center;">0.0</div>	OTHER: <div style="text-align: center;">0.0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <div style="margin-top: 20px;"> <p>*[This project has been subsumed under the projects: Traumatic Coma Data Bank Z01-NS-02516-05) and Stroke Data Bank (Z01-NS-02598-04).]</p> </div>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02498-06 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Observer Agreement Studies\*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Selma C. Kunitz, Ph.D. Chief, Computer BFSB, IRP, NINCDS  
Applications Section

Others: Irene G. Fishman, M.S. Statistician BFSB, IRP, NINCDS  
Christine L. Wolf Programmer BFSB, IRP, NINCDS  
Margaret A. Meadows Statistical Assistant BFSB, IRP, NINCDS  
David Shinar, Ph.D. Psychologist BFSB, IRP, NINCDS

## COOPERATING UNITS (if any)

Depts. of Neurology: Boston Univ. School of Medicine;  
Michael Reese Hospital; N.Y. Neurological Institute; Univ. of Maryland School  
of Medicine. Depts. of Neurosurgery: Univ. of Virginia; Medical College of  
Virginia; Univ. of Texas at Galveston; Univ. of California - San Diego.

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Computer Applications Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL:

0.1

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

To demonstrate that data from the Stroke (Z01-NS-02598-04) and Traumatic Coma Data Banks (Z01-NS-02516-05) are reliable, studies of inter-observer agreement have been conducted. These studies included examination of variation in neurological examination, diagnosis and CT scan reading.

A paper on observer agreement in stroke diagnosis is in press. The paper reports that high levels of agreement in diagnosis of stroke mechanism have been reached among neurologists collaborating in a common research effort. Diagnosis of stroke type was generally more reliable than individual signs and symptoms. A manuscript on observer agreement in CT readings of stroke anatomy has been submitted for publication. In general, levels of agreement were excellent for detection of infarcts and ICH. Substantial agreement was also obtained on whether or not the CT was normal and on indications of small and deep infarcts, superficial and deep infarcts, and aneurysms.

\*[This project has been subsumed under the projects: Traumatic Coma Data Bank (Z01-NS-02516-05) and Stroke Data Bank (Z01-NS-02598-04).]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02599-04 BFSB
PERIOD COVERED October 1, 1985 through September 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Behavioral Factors Influencing Recovery from Stroke		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Selma C. Kunitz, Ph.D. Chief, Computer Applications Section BFSB, IRP, NINCDS		
COOPERATING UNITS (if any) Boston University (Philip Wolf); University of Maryland (Tom Price); Michael Reese Medical Center (Lou Caplan); University of South Alabama (Jay Mohr)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Computer Applications Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.10	PROFESSIONAL 0.10	OTHER 0.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  In order to better comprehend the factors influencing recovery from stroke, <u>behavioral factors</u> were studied, utilizing the first 436 patients of the Stroke Data Bank population (N01-NS-2-2302, N01-NS-2-2398, 2399, N01-NS-5-2384). Specifically, two dimensions of social support were examined with respect to stroke outcome. The two dimensions were source (family and institutional) and type (affective and instrumental). Patients were stratified by stroke severity. Definition of outcome included Activities of Daily Living (ADL) and social functioning. Data collection for this project began in July, 1983. Outcome differences at three months were associated with family support and friend support, depending upon stroke severity. This project has been discontinued and is considered completed.		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02408-08 BFSB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Epidemiologic Research with Clinical Data Banks*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Irene G. Fishman, M.A.  Others: Selma C. Kunitz, Ph.D. Christine L. Wolf Margaret Meadows David Shinar, Ph.D.	Statistician  Chief, CAS Programmer Statistical Assistant Psychologist	BFSB, IRP, NINCDS  BFSB, IRP, NINCDS BFSB, IRP, NINCDS BFSB, IRP, NINCDS BFSB, IRP, NINCDS
COOPERATING UNITS (if any) Depts. of Neurology: Boston Univ. School of Medicine; Michael Reese Hospital; N.Y. Neurological Institute; Univ. of Maryland School of Medicine. Depts. of Neurosurgery: Univ. of Virginia; Medical College of Virginia; Univ. of Texas at Galveston; Univ. of California - San Diego.		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Computer Applications Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>Work on determining which <u>epidemiologic approaches</u> are most appropriate for use with clinical data banks was begun in conjunction with the Pilot Stroke and Traumatic Coma Data Bank Networks and has continued with the full phases of these projects (N01-NS-2-2302, 2398, 2399, N01-NS-5-2384; N01-NS-3-2339-2342).</p> <p>Work completed in FY '86 included publication of a quality assurance study on the validity of a depression symptoms scale which was developed for epidemiology surveys (CES-D), for use with the Stroke Data Bank in the assessment of the incidence and severity of depression in Stroke Data Bank patients. A reliability and validity study of the telephone assessment of Activities of Daily Living for stroke patients has been completed and a paper describing this work is in preparation.</p> <p>*[This project has been subsumed under the projects: Traumatic Coma Data Bank (Z01-NS-02516-05) and Stroke Data Bank (Z01-NS-02598-04).]</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02590-04 BFSB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Statistical Studies on the Stroke Data Bank		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Mary A. Foulkes, Ph.D. Senior Staff Fellow BFSB, IRP, NINCDS  Others: James M. Dambrosia, Ph.D. Chief, Mathematical Statistics Section BFSB, IRP, NINCDS		
COOPERATING UNITS (if any) Departments of Neurology: Boston University Medical Center, Michael Reese Hospital, Neurological Institute-Columbia University, and University of Maryland		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.30	PROFESSIONAL: 0.25	OTHER: 0.05
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             This project currently includes four studies each of which is a component of the Stroke Data Bank or its precursor, the Pilot Stroke Data Bank. The studies are: (1) <u>Evolving Stroke</u>. Using demographic, history, clinical and laboratory data, this study describes the temporal course of <u>stroke-in-evolution</u> and attempts to identify factors that cause or contribute to evolution. (2) <u>Stroke Diagnosis</u>. A set of diagnostic algorithms for stroke classification based on laboratory and clinical findings were developed during the pilot project. The usefulness of the algorithms is being evaluated for differentiating etiology and predicting outcome. Plans for analyses have been formulated. (3) <u>Utility of diagnostic tests</u>. A variety of diagnostic tests (including angiography, CT scanning and noninvasive cardiac and vascular tests) are available for the study of the stroke patient. We intend to investigate the utility of each of these tests in establishing stroke cause and examine the utility of these tests in predicting survival rate, degree of recovery, and risk of stroke recurrence. Study designs and analyses plans have been formulated for this study. (4) <u>Prognostic factors for 30-day mortality</u>. Multiple logistic regression models, one for ischemic stroke and another for intracerebral hemorrhage were used to determine prognostic factors for 30-day mortality. Logistic models were derived using data from the pilot project: 620 ischemic strokes with 52 deaths and 94 intracerebral hemorrhages with 32 deaths. Potential risk factors (112 in all) were initially screened by univariate statistical methods and those screened positive were examined multivariately in the logistic model. These derived models will be cross-validated by examination of their predictive ability on data from the current Stroke Data Bank.           </p>		



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02637-03 BFSB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Stroke and Trauma Program Phase I-II Studies of Stroke Therapies*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: James M. Dambrosia, Ph.D. Chief, Mathematical Statistics Section BFSB, IRP, NINCDS  Others: Robert Richter Mathematician BFSB, IRP, NINCDS Sylvia Edelstein Chief, Data Processing Section BFSB, IRP, NINCDS		
COOPERATING UNITS (if any) Stroke and Trauma Program, NINCDS; University of Pittsburgh; University of S. Alabama; University of Iowa; University of Cincinnati; New York University Medical Center		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 0.8	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>This project includes all aspects of data coordination and management, for studies of <u>interventional therapies</u> for stroke initiated by task orders issued under the aegis of the STP Master Agreement. Currently six studies, each with two clinical centers, are in various stages of operation. A dose escalation study of <u>Naloxone</u> on 27 patients with acute cerebral ischemia has been completed and based on maximum tolerated dose, toxicity, and adverse effects were established. A Phase II study of Naloxone with 38 patients was undertaken and analysis files have been constructed. A pilot study of the benefits of <u>hypervolemic hemodilution</u> (DEXTRAN-40) for the treatment of <u>stroke-in-evolution</u>, and a dose-escalation Phase II study of <u>Nicardipine</u>, a calcium channel blocker, for the prevention of vasospasm following subarachnoid hemorrhage are ongoing.</p> <p>Data management systems have been created for Phase II studies of <u>Heparinoid</u> and <u>Nicardipine</u> for the treatment of acute cerebral ischemia. Patient accrual and data collection for the first study began in May and the other begins in July, 1986. They both will continue patient accrual for approximately one year.</p> <p>*[This project supports the Stroke and Trauma Program contract entitled: Cerebrovascular Clinical Research Master Agreement. The Project Officer is Dr. John Marler.]</p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02483-06 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Predictive Value of the EEG in Febrile Seizures

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jonas H. Ellenberg, Ph.D. Chief

BFSB,IRP,NINCDS

Others: Karin B. Nelson, M.D.

Chief, Cerebral Palsy and  
Other Motor Disorders Sec.

DNB,CDNDP,NINCDS

Deborah G. Hirtz, M.D.

Pediatric Neurologist

DNB,CDNDP,NINCDS

Martha Griswold

Statistician

BFSB,IRP,NINCDS

## COOPERATING UNITS (if any)

Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS;  
Pediatric Clinic, University of Skopje, Yugoslavia (Nikola Sofijanov)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.30

## PROFESSIONAL:

0.10

## OTHER:

0.20

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This population based study will evaluate the significance of the EEG as a predictor for recurrence of seizures in those children who have had a simple febrile convulsion. Outcome with respect to febrile seizure recurrence and afebrile seizure occurrence will be reported. The evolution of the EEG pattern will be described, and patterns will be correlated with the clinical outcome. The clinical study is being carried out in Skopje, Yugoslavia, at the Pediatric Clinic of the University of Skopje.

The study began in FY '82 and will be completed in FY '88. Patient accrual was completed in December, 1984, by which time approximately 400 patients with a febrile seizure, no prior complex or multiple seizures and with a normal or nonspecific abnormal EEG, were registered into the study and began the study protocol and follow-up. An additional 200 patients with a specific abnormal EEG were entered for baseline information and follow-up. Data monitoring, editing and file creation are continuing. Statistical analysis of short-term outcomes and EEG changes will begin in FY '87. Follow-up should be completed on all patients by FY '88.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02411-08, BFSB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Survey of Practice in the Management of Febrile Seizures		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI:           Young Jack Lee, Ph.D.           Mathematical Statistician   BFSB, IRP, NINCDS  Others:   Jonas H. Ellenberg, Ph.D. Chief   BFSB, IRP, NINCDS Deborah G. Hirtz, M.D.   Pediatric Neurologist                           DNB, CDNDP, NINCDS Karin B. Nelson, M.D.   Chief, Cerebral Palsy and                        Other Motor Disorders Sec. DNB, CDNDP, NINCDS		
COOPERATING UNITS (if any) Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.20	PROFESSIONAL: 0.15	OTHER: 0.05
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>           A survey of clinical practice in the management of <u>febrile seizures</u> was conducted using the AMA membership list. The survey questionnaire was sent to a probability sample of 10,000 physicians. The primary data analysis has been completed. From the analysis, it was determined how each medical discipline manages children with febrile seizures. Medical factors that are, in themselves, considered sufficient for chronic treatment or consultation were determined for each <u>medical</u> specialty. Goals of treatment, preferred medicines, whether and why to monitor blood drug levels, and whether and why to hospitalize were analyzed for each specialty. In order to carry out the appropriate analysis, methods for adjusting the effects of concomitant variables for the categorical data analysis were evaluated. The logistic regression was selected as a principal method for the adjustment. The results of the survey indicate that the management of a child with febrile seizures may differ depending on the specialty of the attending physician.         </p> <p>           A paper reporting the major findings has been accepted by the <u>American Journal of Diseases of Children</u>.         </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02594-04 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factors Predictive of Reading and Writing Skills in the Congenitally Deaf\*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Richard F. Raubertas, Ph.D.	Mathematical Statistician	BFSB, IRP, NINCDS
Others:	Christy Ludlow, Ph.D.	Speech Pathologist	CDP, NINCDS
	Judith Cooper, Ph.D.	Speech Pathologist	CDP, NINCDS

## COOPERATING UNITS (if any)

Central Institute for the Deaf, St. Louis, MO (Ann Geers);  
Gallaudet College, Washington, D.C. (Donald Moores)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.2

## PROFESSIONAL:

0.1

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project consists of the statistical and data management aspects of a Communicative Disorders Program contract. Tasks include design of data collection and monitoring procedures, and statistical analysis of study data.

The study will examine factors that may be associated with development of reading and writing skills in the congenitally deaf. Study subjects will comprise three groups of deaf 16- to 17-year-olds, with 65 subjects in each group. Each group will include only subjects who received their preschool language training through one of three approaches: aural-oral, total communication, and American Sign Language. Data will be collected on the audiologic, familial, and educational background of the subjects, and on their present language skills. These data will be examined for their association with present reading and writing skills of the subjects. A pilot study has been completed and the main data collection phase is now in progress.

\*[This project is the BFSB/NINCDS support of the CDP contract study NIH-NINCDS-84-19. The project officer is Dr. Christy Ludlow, CDP/NINCDS.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02638-03 BFSB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Survey of Major Neurological Disorders in Copiah County, Mississippi		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Dallas W. Anderson, Ph.D. Mathematical Statistician BFSB, IRP, NINCDS Others: Bruce S. Schoenberg, M.D., Ph.D. Chief NEB, IRP, NINCDS		
COOPERATING UNITS (if any) University of Mississippi Medical Center, Jackson, MS (Armin F. Haerer)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.50	PROFESSIONAL: 0.40	OTHER: 0.10
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The primary objective of the project was to establish the <u>prevalence</u> of major neurologic and developmental disorders (<u>stroke</u>, <u>epilepsy</u>, <u>cerebral palsy</u>, <u>Parkinson's disease</u>, <u>essential tremor</u>, and <u>dementia</u>) in a well-defined population of southern blacks and whites. A secondary objective was to evaluate certain screening questions for possible use in other morbidity surveys.</p> <p>The background information and methods employed in the study as well as the major findings on prevalence have been published or are in press. A manuscript presenting an overview of the disorders and their frequencies in the Copiah County population has been accepted for publication in the <u>Southern Medical Journal</u>. Another manuscript, on functional disability associated with major neurological disorders, has been submitted for publication. Work is in progress on a manuscript addressing the methodologic issues of the study.</p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02114-13 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Etiology and Natural History of Convulsive Disorders and Cerebral Palsy\*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jonas H. Ellenberg, Ph.D. Chief BFSB, IRP, NINCDS

Others: Karin B. Nelson, M.D. Chief, Cerebral Palsy and Other Motor Disorders Section DNB, CDNDP, NINCDS

Deborah Hirtz, M.D. Pediatric Neurologist DNB, CDNDP, NINCDS

## COOPERATING UNITS (if any)

Cerebral Palsy and Other Motor Disorders Section, DNB, NDP, NINCDS

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.8

## PROFESSIONAL:

0.5

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study examines the relationship between perinatal and early postnatal factors and the occurrence of seizure disorders and cerebral palsy in childhood. The project derives from the data of the Collaborative Perinatal Project, a large prospectively-followed population (approximately 60,000 mothers, with their children followed to seven years of age). The univariate screen of maternal, obstetric and pediatric risk factors, demographic analyses and studies of natural history have been completed. The major focus this fiscal year has been on the multivariate assessment of the data bank which has been substantially completed, including correlation and regression analyses relating to the etiology of both disorders. Final manuscripts in each area have been completed including pre and postnatal predictors of both disorders. This project has been completed.

\*[This study is the BFSB/NINCDS portion of larger studies entitled: Convulsive Disorders Data Analysis Group, and Cerebral Palsy Data Analysis Group. The Principal Investigator for these studies is Dr. Karin B. Nelson, Chief, Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02505-06 BFSB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Headache in Pregnant Women		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Ta-Chuan Chen, Ph.D. Mathematical Statistician BFSB, IRP, NINCDS  Other: Karin Nelson, M.D. Chief, Cerebral Palsy and Other Motor Disorders Sec. DNB, CDNDP, NINCDS  Sylvia Edelstein Chief, Data Processing BFSB, IRP, NINCDS Section		
COOPERATING UNITS (if any) Boston Children's Hospital (Dr. Alan Leviton)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.7	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)  <p>             This project investigates the relationship between <u>migraine headache</u> and other diseases based on the data collected from the large group of gravidae in the Collaborative Perinatal Project. Subgroups of women characterized by the absence and presence of migraine and other recurrent headaches prior to or during pregnancy, have been identified. Characteristics of these subgroups are being investigated on a variety of demographic, sociological, medical and obstetric factors, and the association of headache with other disorders is being examined. Preliminary results have shown that pregnant women with a migraine history had higher rates of smoking and of other symptoms and illnesses than women without a migraine history. Although there is no evidence that cigarette smoking might trigger or exacerbate migraine attacks in these women, smoking might have an additive effect on the association of migraine conditions with heart and thrombotic diseases, some respiratory and allergic diseases and peptic ulcer.           </p> <p>             Children of mothers with a history of migraine appear to have higher incidence of seizures and some infectious and allergic diseases than children born to mothers in the nonmigraine group. More intensive statistical analyses are being carried out to examine the apparent associations.           </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02312-10 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Maternal Infection Study\*

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jonas H. Ellenberg, Ph.D. Chief BFSB, IRP, NINCDS

Other: John L. Sever, M.D., Ph.D. Chief IDB, IRP, NINCDS  
 Martha Griswold Statistician BFSB, IRP, NINCDS  
 Anita Ley Microbiologist IDB, IRP, NINCDS  
 Dorothy Edmonds Clinical Nurse IDB, IRP, NINCDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.15

## PROFESSIONAL:

0.10

## OTHER:

0.05

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analysis of the Collaborative Perinatal Project (CPP) data continues in the area of maternal infection. (The CPP is a prospective study of approximately 60,000 gravidae and the follow-up of their children through the seventh year of life.) The relationship of maternal infection during pregnancy with the later status of the child is being examined using both clinical and serologically-confirmed infections in the mother.

Two primary methodologies have been used, a prospective and a case control approach. A prospective assessment of risk of specified childhood outcome was used for all clinically confirmed infections. Since serological confirmation of all of the common infections occurring during pregnancy on all women in the project would not have been feasible, a case control design has been implemented assessing the titer of 11 antigens in women with abnormal children, in comparison with matched control women with normal children. Special studies of specific infections such as condylomata and toxoplasmosis are in progress or have been completed and a descriptive study of the distribution of titers and frequency of seroconversions by race and age of gravidae for various antigens in a population of pregnant women has been completed. The prospective serological study of toxoplasmosis and its relationship with pregnancy outcome, based on the first 23,000 pregnancies in the study, has shown increases in the risk of deafness, microcephaly and low IQ among children born to women with high maternal antibody to toxoplasmosis. A manuscript reporting the results of the toxoplasmosis study has been accepted for publication.

\*[This study is the BFSB/NINCDS portion of a larger study entitled: Perinatal Infections Causing Damage to the Child - Collaborative Perinatal Project, Z01 NS 00402-30 ID. The principal investigator on the overall study is Dr. John L. Sever, Chief, IDB, IRP, NINCDS.]

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02653-02 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Course and Prognosis Associated with Visual and Hearing Impairment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frances M. Baker, M.D. Psychiatrist/Epidemiologist BFSB, IRP, NINCDS

## COOPERATING UNITS (if any)

Center for Epidemiologic Studies, Division of Biometry and Epidemiology, NIMH  
(Eve Mościcki); Chief, Epidemiology, Demography and Biometry Program, NIA  
(Lon White).

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.10

## PROFESSIONAL:

0.10

## OTHER

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Utilizing the NHANES I data (1971-1975), persons with visual and hearing impairment were divided into several levels of impairment. The NHANES Follow-up Study (1982-1984) was used to determine the specific outcomes. Specific variables considered included the number of hospitalizations between 1971 and 1984, the number and types of associated diagnoses both medical and psychiatric, and the individual's functional level. The focus of this investigation was to describe the course and prognosis associated with visual and hearing impairment and to identify populations at increased risk for deterioration in their functional level. This project has been transferred to NIA and the NINCDS portion has been completed.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02639-03 BFSB

## PERIOD COVERED

September 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antecedents and Consequences of Premature Rupture of Membranes in Pregnancy

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard F. Raubertas, Ph.D. Mathematical BFSB, IRP, NINCDS  
Statistician

## COOPERATING UNITS (if any)

Obstetrics and Gynecology, George Washington University Medical Center  
(Dr. John Grossman and Dr. Goldee Gross)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.0

## PROFESSIONAL:

0.0

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project consisted of the statistical aspects of a study initiated at the George Washington University Medical Center. The primary task included computerization and statistical analysis of study data.

Data were collected on the mothers and infants involved in about 135 cases of premature rupture of membranes (PROM) seen at the GWU Medical Center. Information available included demographic variables, some aspects of the mother's medical history, various aspects of the labor and delivery, and the immediate post-delivery course of the mother and infant. Those areas of particular interest were the demographic composition of the PROM patients, the relationship between PROM and maternal infection during pregnancy, and the relationship between length of interval from PROM to delivery and various post-delivery complications. These complications include intraventricular hemorrhage and respiratory distress syndrome in the infant, and infections in both mother and infant. This study has ended prior to completion due to the departure of Dr. Gross from George Washington University. This study has been completed.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02497-06 BFSB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less: Title must fit on one line between the borders.) Indo-U.S. Study of Head Injury (Phase I)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 60%;">           PI: Selma C. Kunitz, Ph.D. Chief, Computer Applications Section         </div> <div style="width: 35%;">           BFSB, IRP, NINCDS         </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 33%;">           Other: Christine L. Wolf            Ella Maneely         </div> <div style="width: 33%;">           Programmer            Programmer         </div> <div style="width: 33%;">           BFSB, IRP, NINCDS            BFSB, IRP, NINCDS         </div> </div>		
COOPERATING UNITS (if any) University of VA Dept. of Neurosurgery, Charlottesville, VA All-India Institute of Medical Science, New Delhi, India		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Computer Applications Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.02	PROFESSIONAL 0.01	OTHER 0.01
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input checked="" type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>For Phase I of this study, information on <u>head-injured persons</u> has been collected in independent research efforts in Charlottesville, Virginia, and in New Delhi, India. A preliminary review of these data collection efforts indicated significant overlap in the type of information collected. Final analysis has identified differences and similarities in the etiology, treatment and outcome of these head-injured populations, but has indicated that prospective observational studies or clinical trials on head injured patients is feasible.</p> <p>The report on Phase I of the study has been prepared, and will be submitted for publication.</p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02651-02 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Senile Dementia Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frances M. Baker, M.D. Psychiatrist/Epidemiologist BFSB, IRP, NINCDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.15

## PROFESSIONAL:

0.15

## OTHER:

0.00

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In conjunction with the Baltimore site of the Epidemiologic Catchment Area Survey of NIMH, NINCDS funded a detailed dementia workup on those persons in the elderly sample identified with dementing illness, by comprehensive psychiatric examination. The detailed dementia workup included a neurologic examination and comprehensive laboratory studies which included thyroid function tests, electrolytes, BUN and glucose, B12 and folate levels, calcium and phosphorous levels, syphilis serology, urinalysis, chest X-ray, EKG, EEG, and CT scan. The foci of this investigation were (1) to explore the limitations of the Mini-Mental-State-Examination (MMSE) as a screening instrument, (2) to explore which components of the comprehensive dementia workup provide the highest specificity when used as a dementia screening test, (3) to develop a screening model for dementia with a sensitivity and specificity improved beyond that of the MMSE, and (4) to examine the usefulness of this model in other elderly populations. In addition, for the confirmed cases of dementia, additional information was gathered to assess the social and economic impact upon the caregivers.

A review of the data indicated that only 36 confirmed cases of dementia were identified and available for further analysis. The sample size was not adequate to address the four areas of interest and to develop a model for dementia screening beyond the MMSE. After review of the information on social and economic impact, it was determined that the study of the burden on caregivers was also not feasible. A report of the detailed dementia workup of the 36 cases is being prepared.

PROJECT NUMBER
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Z01 NS 02636-03 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Classification of Headache Types Based on Symptomatology and Features

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert Richter	Mathematician	BFSB, IRP, NINCDS
-----	----------------	---------------	-------------------

Other: Frederic D. Weinfeld, Ph.D. Chief, Surveys and BFSB, IRP, NINCDS  
Demographic Studies (No longer at NIH)  
Section

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Surveys and Demographic Studies Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

**TOTAL MAN-YEARS:**

0.1

PROFESSIONAL:

0.15

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Three studies of headache features in migraine, cluster and tension headaches were developed based on the data collected from a feasibility study for a survey of types of headache (Z01 NS 02404-07). The feasibility study involved 243 patients from four headache clinics. The first study used four group discriminant analysis to develop statistically a parsimonious set of headache features and symptoms which could be used to correctly classify a high percentage of patient headaches into one of the four headache types of common migraine, classical migraine, cluster or tension headache. A second study using factor analysis, on the combined group of headaches, attempted to isolate patterns of symptoms and features of headaches which mirror clinical descriptions of the four headache types above. The study yielded indeterminate results. The third study, describes for each headache type, the frequency of and interrelationships among headache symptoms and features, and relates precipitating factors such as eyestrain, menstruation, etc., to these patterns. No further work is planned in these areas, and the project is completed.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02494-06 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Prevalence of Multiple Sclerosis in Colorado

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Herbert M. Baum, Ph.D. Demographer

BFSB, IRP, NINCDS  
(No longer at NIH)

Others: None

## COOPERATING UNITS (if any)

The Rocky Mountain Multiple Sclerosis Center, University of Colorado School of Medicine

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Surveys and Demographic Studies Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.0

PROFESSIONAL:

0.0

OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Rocky Mountain Multiple Sclerosis Center is one of a few centers devoted solely to the care of patients with multiple sclerosis, and is the only center of its type in the State of Colorado. Using records from the Center, the local chapter of the National Multiple Sclerosis Society, hospital records, and physician records we estimated the prevalence of multiple sclerosis for Weld and Larimer Counties, after accounting for duplicate cases.

Crude point prevalence for the two-county region was 84 per 100,000. Methodological results revealed that the highest yield sources of cases were the MS service organizations and the neurology practice chart reviews. Prevalence surveys which neglect these sources may underestimate MS prevalence by as much as 20 to 40%.

A manuscript "Higher than expected prevalence of multiple sclerosis in Northern Colorado: Dependence on methodologic issues," has been accepted for publication. No further work is planned by BFSB on this project.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02504-06 BFSB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Epidemiological Study of Pain		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Ta-Chuan Chen, Ph.D. Mathematical Statistician BFSB, IRP, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The purpose of this project is to evaluate the overall and <u>age-specific incidence rates</u> of various <u>chronic pain syndromes</u> by developing a statistical technique to estimate incidence rates from age of onset data. The technique developed uses available estimates of age of onset data for headache and approximates incidence rates by superimposing the age of onset rates onto the age distribution of the given population. The incidence rates of disabling and/or severe headache were evaluated with data obtained from a Midwest non-clinical population survey.</p> <p>The validity of this procedure was evaluated by comparing the results with the incidence of disabling headache estimated from the British Second National Study of Morbidity Statistics. A report on this work has been prepared and will be submitted for publication.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02517-05 BFSB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Methodology for the Measurement of Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ta-Chuan Chen, Ph.D. Mathematical Statistician BFSB, IRP, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the statistical problems involved in the measurement of experimental and clinical pain. (1) A study has been conducted to investigate the statistical technique used in deriving psychophysical measurements of pain. A report has been prepared for this work dealing with the interrelationship of sensory-decision-theory measures such as  $d'$  and  $\beta$  and nonparametric measurement indices, such as  $p(A)$ , Hodo's percent bias and MacNicol's index of response bias,  $\beta$ . The investigation of this part of the work is completed. The report of this study was presented at the 4th World Congress on Pain in 1984. A manuscript is in preparation. (2) A study of statistical quantification of the temporal characteristics of persistent, episodic pain such as migraine headache is currently being developed. A group of measurements for this type of pain has been selected for investigation.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02654-01 BFSB									
PERIOD COVERED March 15, 1986 through September 30, 1986											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Incidence of Mental Retardation (MR) in Olmstead County											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Frances M. Baker, M.D.</td> <td style="width: 33%;">Psychiatrist/ Epidemiologist</td> <td style="width: 33%;">BFSB, IRP, NINCDS</td> </tr> <tr> <td>Others: Bruce S. Schoenberg, M.D., Ph.D.</td> <td>Chief</td> <td>NEB, IRP, NINCDS</td> </tr> </table>			PI: Frances M. Baker, M.D.	Psychiatrist/ Epidemiologist	BFSB, IRP, NINCDS	Others: Bruce S. Schoenberg, M.D., Ph.D.	Chief	NEB, IRP, NINCDS			
PI: Frances M. Baker, M.D.	Psychiatrist/ Epidemiologist	BFSB, IRP, NINCDS									
Others: Bruce S. Schoenberg, M.D., Ph.D.	Chief	NEB, IRP, NINCDS									
COOPERATING UNITS (if any) Neuroepidemiology Branch, IRP, NINCDS; Mayo Clinic, Statistical Section (Leonard Kurland, Chief)											
LAB/BRANCH Biometry and Field Studies Branch											
SECTION Mathematical Statistics Section											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892											
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews		
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither									
<input type="checkbox"/> (a1) Minors											
<input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 20px;">             In conjunction with Dr. Leonard Kurland of the Mayo Clinic in Rochester, Minnesota, the birth records and school records of all Olmstead County residents born in 1960 and 1961 will be reviewed. All persons with mental retardation will be identified from these sources. <u>Mental retardation</u> (MR) is defined for the purpose of this study, as a fixed cognitive deficit which occurred before age 12 with some impairment of social adaptation. In addition to the specification of the incidence and identification of risk factors for MR in this birth cohort, <u>service utilization</u> by the MR cases will be assessed. Specific services which will be assessed will include medical (psychiatric, neurologic, gynecologic, and surgical), social (group homes, sheltered workshops, and rehabilitative programs), and educational (special program or projects and institutional placements).           </p>											

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02655-01 BFSB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Severe Dementia in Rural Ecuador

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frances M. Baker, M.D.

Psychiatrist/

BFSB, IRP, NINCDS

Epidemiologist

Others: Bruce S. Schoenberg, M.D., Ph.D.

Chief

NEB, IRP, NINCDS

## COOPERATING UNITS (if any)

Neuroepidemiology Branch, IRP, NINCDS

## LAB/BRANCH

Biometry and Field Studies Branch

## SECTION

Mathematical Statistics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL:

0.3

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In conjunction with Dr. Marcello Cruz, Professor of the Catholic University in Quito, Ecuador, the feasibility of completing a study of the prevalence of severe dementia in rural Ecuador was explored. A three phase approach was designed with the successful completion of the preceeding stage as the criteria for advancing to the next stage.

The first phase involved the development of screening instruments and an assessment of the sensitivity and specificity of these instruments to identify patients with severe dementia. When the screening instruments were tested in Catamayo, Ciudad a rural city in Ecuador, the field results were inconclusive and significant problems in field activities occurred.

As the first phase was unsuccessful, it was decided not to implement further phases of this project. This project is completed.

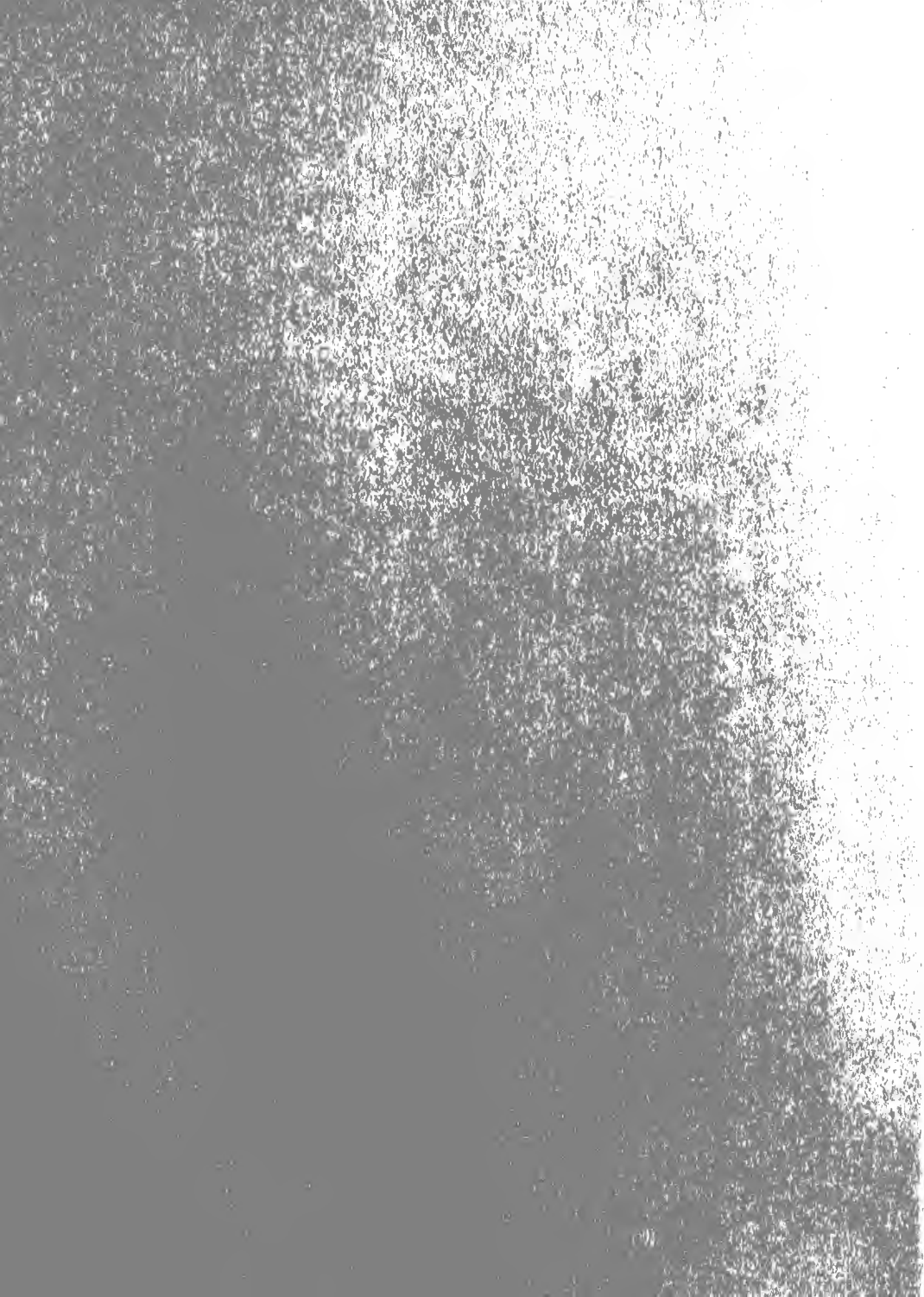
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NS 02506-06 BFSB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Antibody Titers in Macacas on Cayo Santiago		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Statistician (to be assigned when data become available) BFSB, IRP, NINCDS		
Others: William T. London, D.V.M. Chief, Experimental Pathology Section IDB, IRP, NINCDS		
COOPERATING UNITS (if any) Infectious Diseases Branch, IRP, NINCDS; Caribbean Primate Research Center, University of Puerto Rico (Matthew J. Kessler, Project Director)		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland, 20892		
TOTAL MAN-YEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>This project will test for the presence of several viral antibodies in <u>adult and juvenile Macacas</u> on Cayo Santiago, Puerto Rico. This has been a closed colony of Rhesus monkeys since 1938. A serological screen carried out in the early 1950's indicated the presence of antibody to SV 40 (46%), herpes B (27%) and <u>measles</u> virus (80%) of the animals in the colony. Three additional antigens have been added to the screen. They are: Rhesus CMV, simian retrovirus D (SRV I) and simian T-Lymphotropic virus type III (STLV III). The objective will be to determine after some 40 years as a closed colony, if herd infection to the three previously studied antigens has been lost and would, therefore, provide an animal population useful for the testing of related strains of viruses. If this colony is shown to be serologically negative for the other three simian viruses, CMV, SRV I and STLV III, then animals from this colony could be used in the study of these simian diseases as models for related human diseases.</p>		





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02591-04 BFSB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Reye's Syndrome Study*		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Young Jack Lee, Ph.D. Mathematical Statistician BFSB, IRP, NINCDS  Others: Anita Chu Expert IDB, IRP, NINCDS		
COOPERATING UNITS (if any) Infectious Diseases Branch, IRP, NINCDS		
LAB/BRANCH Biometry and Field Studies Branch		
SECTION Mathematical Statistics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.00
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The Infectious Diseases Branch studied salicylate metabolism, other clinical chemistries and <u>histocompatibility antigens</u> in families with <u>Reye's Syndrome</u> patients who have completely recovered from the syndrome. BFSB was responsible for all statistical components of the study including design, data analysis and statistical modeling of the clinical chemistry data.</p> <p>Five survivors and their unaffected family members were studied. This study showed significantly higher antibody levels to Influenza A and varicella, further supporting the importance of these viral infections in the etiology of the syndrome. It did not show an association between RS and 1) abnormal salicylate metabolism, 2) abnormal helper to suppressor T cell ratios and lymphocyte stimulation responses, 3) specific HLA type, and 4) permanent neuropsychologic sequelae. A paper has been accepted for publication.</p> <p>* [This project has been subsumed under: Intramural Statistical Collaboration and Consultation (Z01-NS-02652-02)]</p>		





## ANNUAL REPORT

October 1, 1985 through September 30, 1986  
Developmental and Metabolic Neurology Branch  
National Institute of Neurological and Communicative Disorders and Stroke

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## ANNUAL REPORT

October 1, 1985 through September 30, 1986  
Developmental and Metabolic Neurology Branch, IRP  
National Institute of Neurological and Communicative Disorders and Stroke

Roscoe O. Brady, M.D., Chief

Principal activities of the Branch concern the following areas of investigation: 1. Sphingolipid and mucopolysaccharide synthesis and catabolism and elucidation of enzymatic abnormalities in human metabolic disorders. 2. Clinical studies of neurogenetic diseases. 3. Production of cellular and animal models of disorders of metabolism. 4. Elucidation of the molecular basis of lysosomal storage disorders. 5. Development of therapy for patients with heritable diseases. 6. Transmembrane signalling and the role of glycoconjugates in this process. 7. The role of glycolipids and glycoproteins in the development of the nervous system, autoimmune phenomena, and in demyelinating diseases.

### I. HEREDITARY METABOLIC DISORDERS

#### A. Type C Niemann-Pick Disease.

The discovery of impaired esterification of exogenous cholesterol in Type C Niemann-Pick disease has been advanced in three major directions. The first is the practical application of this finding for the development of specific and sensitive tests for the diagnosis of homozygotes and the identification of heterozygous carriers of this disorder. The procedure is based on the use of the fluorogenic dye filipin that forms an intensely fluorescent product when complexed with free cholesterol. We expect that this test will be equally useful for the prenatal diagnosis of this disorder. The second advance is the demonstration that there is impaired regulation of two key processes involved in the regulation of the intracellular disposition of cholesterol. There is less down-regulation of specific low-density lipoprotein binding which is responsible for the endocytosis of LDL-cholesterol in cultured skin fibroblasts from Type C Niemann-Pick disease patients than in cells from normal individuals. This implies constant ingress of cholesterol into cells which normally would shut down this mechanism. There is slower than normal up-regulation of acyl cholesterol acyl CoA transferase, the enzyme that catalyzes the intracellular esterification of the free cholesterol within cells. This delay could be in part responsible for the excess of free cholesterol in patients' cells. Thirdly, we now have available cells derived from patients with Type D Niemann-Pick disease which is a genetic isolate in Nova Scotia with many of the features of Type C Niemann-Pick disease. We are therefore in position to determine whether there is a biochemical relationship between these phenotypes of Niemann-Pick disease.

### II. CLINICAL INVESTIGATIONS OF NEUROGENETIC DISEASES

Etiologically nondefined neurogenetic disorders, as observed in patients

seen in the Section on Clinical Investigations and Therapeutics, frequently present an opportunity for significant biochemical and molecular genetic studies. Examples of illnesses currently under investigation on the ward and in the clinic include tetrahydrobiopterin-deficient forms of dystonia, X-linked movement disorders, and hypersecreters of dolichols in the urine. Analysis of materials derived from these and other patients provide insight into the cause and the abnormal biochemistry in neurogenetic disorders of unknown etiology.

### III. MODELS OF HUMAN LYSOSOMAL STORAGE DISORDERS

#### A. Biochemical Models of Gaucher's disease.

A model of Gaucher's disease has been developed through the synthesis of glucothiocerebroside, an analog of glucocerebroside in which the oxygen atom of the glycosidic bond between glucose and ceramide has been replaced with an atom of sulfur. This compound is completely refractory to hydrolysis by glucocerebrosidase and it should be useful for determining the organ and tissue distribution of the stored lipid and pathogenic changes in rodent and murine models of Gaucher's disease. We have continued our investigations with the L-enantiomorphous analog of glucocerebroside where the normal molecule of D-glucose has been replaced with L-glucose. This cerebroside has physical qualities characteristic of the natural glucocerebroside but it is not susceptible to enzymatic breakdown.

Another important advance was the development of a new procedure for the synthesis of conduritol B epoxide. This compound is a potent inhibitor of glucocerebrosidase and causes the accumulation of glucocerebroside in animals injected with this material. The new method permits the synthesis of conduritol B epoxide with exceptionally high specific radioactivity so that the catalytically active site of glucocerebrosidase can be identified and insight obtained in the amino acid changes that occur in the mutated enzymes in patients with Gaucher's disease.

#### B. Biochemical Model of Krabbe's disease.

Because of the usefulness of L-glucosylceramide to examine metabolic pathways in models of Gaucher's disease, we synthesized L-galactosylceramide to develop a model of Krabbe's disease which is characterized by a deficiency of galactocerebroside- $\beta$ -galactosidase. This analog of galactocerebroside is refractory to enzymatic hydrolysis and it should be useful for investigating pathological aspects of Krabbe's disease in animal models and in myelinating tissue cultures in vitro.

#### C. Pharmacological Model of Hurler's disease.

We have continued our investigations on the reversibility of mucopolysaccharide accumulation in the suramin-induced rodent model of Hurler's disease. Tissues such as the liver show rapid reduction of glycoconjugates after cessation of suramin whereas in the kidney, mucopolysaccharide levels are still many fold over normal six months after the drug is stopped. These findings have important implications regarding therapeutic approaches to metabolic storage disorders and indicate that large



variations in tissue responses to treatment strategies should be anticipated.

#### D. Canine Hurler's disease.

The Branch has continued a collaborative study with investigators at the University of Tennessee with spontaneously mutated Plott hounds that resemble mucopolysaccharidosis Type I (Hurler syndrome) in humans. Bone marrow transplantation has been performed on affected animals and the biochemical responses were monitored by DMNB. A major unexpected finding of this work was that there were definite indications of decreased concentrations of mucopolysaccharides within the CNS of the transplanted animals. This finding has extraordinarily important implications for the treatment of humans with Hurler's syndrome and other metabolic storage disorders since most investigators did not believe that bone marrow transplantation would lead to beneficial changes in the nervous system.

### IV. MOLECULAR GENETICS OF LYSOSOMAL STORAGE DISORDERS

The gene for ceramidetrihexosidase, the enzyme lacking in patients with Fabry's disease, has been cloned by investigators in the section on Molecular and Medical Genetics. This is a major accomplishment concerning (1) the acquisition of knowledge of the molecular pathology in Fabry's disease, (2) the possibility of producing ceramidetrihexosidase by recombinant DNA technology, (3) the development of new diagnostic procedures involving DNA restriction fragment length polymorphisms in patients and carriers of this disorder, (4) accurate gene mapping, and (5) potential therapeutic applications including considerations of gene engineering or replacement. To this end, retroviral vector test systems have been developed to establish the feasibility of transfer of the ceramidetrihexosidase gene. Furthermore, since the enzymes involved in Niemann-Pick disease and Hurler's disease have now been purified, it is expected that the genes for these enzymes will soon be isolated. These developments will permit examination of the chromosomal localization, identification of variations in the genetic code in patients, development of novel diagnostic tests, and investigation of the factors involved in the expression of these genes.

### V. ENZYME REPLACEMENT THERAPY

A clinical trial of enzyme replacement has been carried out in Gaucher's disease using purified human placental glucocerebrosidase whose oligosaccharide residues were modified so that they terminate with the hexose mannose to target the enzyme to cells of the monocyte/macrophage system. Administration of this enzyme caused consistent hematologic improvement in a young patient with Gaucher's disease. Withdrawal of the enzyme resulted in worsening of his clinical parameters and reinstitution again brought about improvement. The study will be continued in order to document the consistency of these responses in the recipient and in other Gaucher patients.

### VI. MEMBRANE RECEPTORS FOR PHYSIOLOGICAL AND ENVIRONMENTAL SIGNALS

#### A. Role of Gangliosides as Biotransducers of Growth Regulation.

It has been shown that the B or binding subunit of cholera toxin, which

reacts only with ganglioside GM1 on the cell surface, is a potent mitogen for lymphocytes. This discovery has been extended to normal and transformed mouse 3T3 fibroblasts. 3T3 cells transformed by the ras oncogene expressed lower quantities of complex gangliosides GM1 and GD1a on their cell membranes compared with normal contact-inhibited 3T3 cells. In addition, rapidly dividing, as well as mitogen-stimulated 3T3 cells, had lower levels of surface GM1 and GD1a than their confluent, quiescent counterparts. To explore the role of surface gangliosides in cell growth, quiescent 3T3 cells were exposed to the B subunit which stimulated cell growth. The B subunit also potentiated the effects of other mitogens such as epidermal growth factor, platelet-derived growth factor and insulin. In contrast, the B subunit inhibited the growth of transformed 3T3 cells as well as that of rapidly dividing normal 3T3 cells. Thus, gangliosides appear to be membrane transducers of both positive and negative signals that regulate cell growth.

## B. Regulation of Receptor-Coupled Adenylate Cyclase.

### 1. Agonist-mediated desensitization of mammalian cells.

Exposure of mammalian cells to isoproterenol resulted in a desensitization of agonist-stimulated adenylate cyclase activity and a sequestration of  $\beta$ -adrenergic receptors into a lighter density membrane fraction inaccessible to hydrophilic antagonists. When the cells were washed free of agonist, resensitization and reappearance of the sequestered receptors occurred. Pretreatment of the cells with concanavalin A blocked sequestration but not desensitization. Using membrane fusion, the receptors were transferred to a foreign adenylate cyclase and their function measured. A reduction in receptor function occurred during desensitization and in the absence of sequestration. Upon resensitization, receptor function was recovered. Thus, the key event in agonist-mediated desensitization is a reduction in receptor function.

### 2. Characterization and solubilization of D-1 dopamine receptors.

Exposure of rat striatal membranes to N-ethylmaleimide (NEM) caused a loss of D-1 dopamine receptors. D-1 specific agonists or antagonists, respectively, fully and partially protected the receptors from inactivation by NEM. Upon transfer of the receptors to a foreign adenylate cyclase by membrane fusion, agonist- but not antagonist-protected D-1 receptors remained functional as measured by agonist stimulation of the cyclase. The D-1 receptor has been solubilized and the selective protection by agonist is being used as a means to purify the receptor.

## C. Structure-Function Relationships of Choriogonadotropin (hCG).

Deglycosylated human choriogonadotropin (DG-hCG) is a potent antagonist of hCG. When DG-hCG is bound to hCG-receptors on murine Leydig tumor cells, addition of anti-hCG reverses the antagonism and results in stimulation of adenylate cyclase. Rabbit antibodies raised to DG-hCG cross-reacted with hCG. When the antiserum was purified on an hCG-agarose affinity column, only the non-absorbed fraction reacted with DG-hCG and did not reverse its antagonism. In contrast, the absorbed antibodies reacted preferentially with the  $\beta$ -subunit of hCG and DG-hCG, reversed the antagonism of DG-hCG and this

reversal was blocked by prior incubation of the antibodies with the  $\beta$ - but not the  $\alpha$ -subunits. Thus, epitopes on the  $\beta$ -subunit may be important for determining whether the hormone is an agonist or antagonist.

## VII. MYELINATION AND DEMYELINATING DISORDERS

### A. Myelin-associated Glycoprotein (MAG) and Other Proteins in Dysmyelinating Mutants.

The localization of MAG in periaxonal oligodendroglia and Schwann cell membranes suggests a critical role for MAG in glia-axon interactions during the formation and maintenance of myelin. Correlation of the loss of MAG with the breakdown of the normal Schwann cell-axon junction in the quaking mutant provided strong evidence that MAG functions in maintaining the normal Schwann cell-axon junction. We have now completed a detailed quantitative study of MAG and other myelin proteins in a wide range of dysmyelinating mutants including quaking mice, jimpy mice, trembler mice, mice with a cholesterol storage disorder, myelin-deficient rats and shaking pups. A general finding in all of the mutants is that MAG is not decreased as much as the proteins of compact myelin; e.g., myelin basic protein (MBP), presumably because the mutants are more deficient in compact myelin than the periaxonal glial membranes in which MAG is localized. In two of the mutants, quaking and trembler, MAG has an abnormally high apparent molecular weight and this may contribute to the pathological changes in these mutants. In jimpy mice and myelin-deficient rats, proteolipid protein (PLP) could not be detected, suggesting that the PLP gene that was recently shown to be on the X-chromosome could be affected directly in these sex-linked recessive mutations.

### B. Glycolipid Antigens in Autoimmune Neuropathy.

Peripheral neuropathy associated with a monoclonal IgM antibody that reacts with carbohydrate epitopes in MAG and other glycoconjugates of the peripheral nervous system is becoming an increasingly important aspect of clinical neurology. The Section on Myelin and Brain Development has identified over 30 patients with this condition from sera screened in our laboratory alone, and numerous other patients with this condition have been detected in medical centers around the world. It has now been established that the principal other glycoconjugates of peripheral nerve sharing carbohydrate epitopes with MAG and reacting with these human antibodies are some 20 to 26K dalton glycoproteins of myelin and sulfated glucuronyl paragloboside (SGPG). The monoclonal anti-MAG IgM in all of these patients reacts with both of these glycoconjugates. Experiments with chemically modified derivatives of SGPG have shown that there is idiotypic heterogeneity with regard to the precise chemical requirements for antibody binding. About 60 percent of patients with IgM gammopathy and neuropathy have anti-MAG and hence anti-SGPG, antibodies. Monoclonal IgM antibodies in over half of the remaining patients react with other acidic glycolipid antigens. These antigens include gangliosides GM1, GM2, GD3, GD1b and GT1b. Therefore, glycolipid antigens have been detected for more than 80 percent of the paraproteins that we have examined from patients with neuropathy associated with IgM gammopathy. This suggests that glycolipids may be especially important target antigens in this type of peripheral nerve disease. Furthermore, we have detected relatively high titers of anti-ganglioside

antibodies in some patients with acute Guillian-Barre syndrome, also indicative of the involvement of glycolipid antigens in the pathogenesis of demyelination in the peripheral nervous system.

### C. MAG in Multiple Sclerosis.

Quantitative analyses of MAG and other myelin proteins in a new group of postmortem multiple sclerosis brain specimens extended earlier biochemical and immunocytochemical studies demonstrating that MAG was reduced more than MBP in periplaque regions by showing that MAG is also reduced significantly more than PLP. Immunoblotting experiments on the affected regions of multiple sclerosis brain revealed that in many cases more than 50 percent of the MAG is in the form of its proteolytic derivative, dMAG, whereas control white matter samples contain very little dMAG. dMAG is about 10,000 daltons smaller than intact MAG and is formed by a neutral protease endogenous to myelin sheaths. The activity of this protease is higher in myelin from multiple sclerosis brain than in control myelin. Furthermore, dMAG is not so tightly anchored to membranes as the intact glycoprotein. It is easily released from membranes and appears in the cerebrospinal fluid. A likely reason for the early loss of MAG from periplaque regions in multiple sclerosis is the conversion of MAG to dMAG by the elevated protease activity and its subsequent release into extracellular fluids. A possible consequence of this effect would be the disruption of oligodendrocyte-axon interactions. These studies provide important insight into pathogenetic processes in multiple sclerosis.

## CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch  
Intramural Research Program, NINCDS  
October 1, 1985 through September 30, 1986

Contractor: GENZYME CORPORATION, BOSTON, MA. (NO1-NS-3-2351)

Title: Preparation of Glucocerebrosidase from Human Placental Tissue

Contractor's Project Director: Henry E. Blair

Current Annual Level of Support: \$409,094

Objectives: To isolate human placental glucocerebrosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Gaucher's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental glucocerebrosidase in sufficient purity and specific catalytic activity so that it can be safely administered to patients with Gaucher's disease. The intravenous infusion of this enzyme appears to have retarded the progression of enlargement of the spleen and liver in several patients with this disorder, stabilized their blood platelet count, and caused an improvement in the general health and growth patterns of the recipients.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy to treat human diseases. If the results indicated in the preceding paragraph can be confirmed and extended, an unprecedented feat will have been accomplished regarding human genetic diseases.

Proposed Course of the Contract: We are investigating procedures to stabilize and target the enzyme to the specific cells in which toxic quantities of lipid accumulate. When a sufficient quantity of the appropriately modified enzyme is available, we shall examine its efficiency in patients. We shall also continue to attempt to develop methods to deliver the enzyme to the central nervous system for the treatment of patients with the neuropathic forms of the disorder.

## CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch  
Intramural Research Program, NINCDS  
October 1, 1985 through September 30, 1986

Contractor: WEIZMANN INSTITUTE OF SCIENCE (N01-NS-3-2349)

Title: Production of Radiolabeled Glycolipids and Other Sphingolipid Derivatives.

Contractor's Project Director: Ora Goldberg, Ph.D.

Current Annual Level of Support: \$76,133

Objectives: To prepare glucocerebroside, sphingomyelin, and ceramidetrihexoside labeled with  $^{14}\text{C}$  in critical portions of the molecule for diagnostic tests for Gaucher's disease, Niemann-Pick disease, and Fabry's disease.

Major Findings: The Weizmann Institute of Science has extensive and recognized expertise in the chemical synthesis of sphingolipids. Procedures have been developed to incorporate radioactive carbon- $^{14}$  into specific portions of sphingolipid molecules. These compounds are used to diagnose patients with the sphingolipid storage disorders listed above, to identify heterozygous carriers of these conditions, to diagnose these disorders prenatally, and to monitor enzyme isolation procedures for glucocerebrosidase, sphingomyelinase, and ceramidetrihexosidase.

Significance to Biomedical Research and to the Program of the Institute: The ability to diagnose patients, identify heterozygotes, and monitor pregnancies at risk for sphingolipid storage disorders represents major contributions to the control of the incidence of these diseases. These procedures are in wide use at the present time.

Proposed Course of the Contract: The contractor will provide radioactive sphingolipids necessary for diagnostic tests and for enzyme purification procedures. Analogues of sphingolipids will be prepared for the development of animal models of the human disorders. Sphingolipid derivatives will be synthesized for use as ligands in affinity column chromatography to expedite and improve the isolation of sphingolipid hydrolases.

## CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch  
Intramural Research Program, NINCDS  
October 1, 1985 through September 30, 1986

Contractor: GENZYME CORPORATION, BOSTON, MA. (N01-NS-3-2346)

Title: Preparation of Ceramidetrihexosidase from Human Placental Tissue

Contractor's Project Director: Henry E. Blair

Current Annual Level of Support: \$104,005

Objectives: To isolate human placental ceramidetrihexosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Fabry's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental ceramidetrihexosidase in sufficient purity and specific catalytic activity so that it can be safely administered to patients with Fabry's disease. The contractor has developed a procedure to remove pyrogen(s) that previously prevented administration of large quantities of ceramidetrihexosidase to patients.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy to treat human diseases. If salutary clinical results can be obtained, an extraordinary milestone will have been accomplished regarding this type of a human genetic disease.

Proposed Course of the Contract: We have begun experiments to increase the half-life of this enzyme in the blood stream of recipients since our investigations indicate that the clearance of accumulated ceramidetrihexoside was probably carried out in circulating leukocytes in patients with Fabry's disease. We expect to reinitiate enzyme replacement therapy in patients with Fabry's disease and we shall examine the effectiveness of the enzyme with regard to clearance of accumulated ceramidetrihexoside in the liver and in the blood, and monitor clinical responses to this potential therapeutic agent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 00815-26 DMN
PERIOD COVERED      October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Metabolism of Complex Lipids of Nervous Tissue		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	R. O. Brady, M.D., Chief, DMN	DMN      NINCDS
OTHERS:	P. G. Pentchev, Ph.D., Biochem.	DMN      NINCDS
	A. E. Gal, Ph.D., Organic Chemist	DMN      NINCDS
	T. Tokoro, M.D., Visiting Fellow	DMN      NINCDS
	J. M. Quirk, Biochemist	DMN      NINCDS
	M. Comly, Biologist	DMN      NINCDS
	H. S. Kruth, Ph.D. Sen. Invest.	EA, IR      NHLBI
COOPERATING UNITS (if any)      Laboratory of Experimental Atherosclerosis, NHLBI		
LAB/BRANCH      Developmental and Metabolic Neurology Branch		
SECTION      Enzymology and Genetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland      20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>1. The metabolic defect in patients with <u>Type C Niemann-Pick disease</u> has now been established as a defect in esterification of cholesterol supplied to cells via the low-density lipoprotein (LDL) receptor or by fluid phase pinocytosis. This discovery has been extended to the development of a <u>diagnostic test</u> for the identification of <u>homozygotes</u> and for the detection of <u>heterozygous carriers</u> of this trait. The molecular defect in this disorder appears to be a coordinate failure to down-regulate LDL receptors on the plasma membrane of cells and the up-regulation of acyl cholesterol acyl CoA transferase, the enzyme that catalyzes the intracellular esterification of cholesterol.</p> <p>2. Other work has centered on the use of <u>non-metabolizable analogues of glucocerebroside</u> and <u>galactocerebroside</u> to examine the organ and tissue disposition and excretion of these lipids that accumulate in <u>Gaucher's disease</u> and <u>Krabbe's disease</u>, respectively. A significant portion of these analogues appears in the bile of experimental animals and provides a reasonable explanation for the lack of accumulation of such lipids in hepatocytes in patients with these disorders.</p>		
10 DMN/IRP		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01309-21 DMN

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. H. Fishman, Ph.D., Chief, Membrane Biochemistry Section, DMN, NINCDS  
OTHERS: S. Spiegel, Ph.D., Visiting Fellow, DMN, NINCDS  
G. Matyas, Ph.D., Staff Fellow, DMN, NINCDS  
R. O. Brady, M.D., Branch Chief, DMN, NINCDS

## COOPERATING UNITS (If any)

Laboratory of Cellular Metabolism, NHLBI  
Laboratory of Kidney and Electrolyte Metabolism, NHLBI  
Laboratory of Molecular Biology, NCI

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Membrane Biochemistry Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.4

## PROFESSIONAL:

2.4

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gangliosides appear to be important recognition molecules on the cell surface and have been implicated as receptors for certain bacterial toxins and viruses. Little is known, however, about the normal physiological role(s) of these plasma membrane components. We found that NIH 3T3 cells transformed by the *ras* oncogene expressed lower amounts of complex gangliosides GM1 and GD1a on the cell surface compared to normal contact-inhibited NIH 3T3 cells. Similar results were obtained with cells transformed by several variants of *ras* including those derived from human tumors. In addition, rapidly dividing as well as mitogen-stimulated NIH 3T3 cells had lower levels of surface GM1 and GD1a than their confluent, quiescent counterparts. To explore the role of surface gangliosides in cell growth, we used the B or binding subunit of cholera toxin as a specific probe for surface GM1. The only known function of the B subunit, which is multivalent, is to bind to the oligosaccharide chain of GM1. Exposure of quiescent 3T3 cells to the B subunit resulted in a proliferative response as measured by increased DNA synthesis and cell numbers. The B subunit potentiated the effects of other mitogens such as epidermal growth factor. In contrast, the B subunit inhibited the growth of transformed 3T3 cells as well as that of rapidly dividing normal 3T3 cells. Thus, gangliosides may play a role as membrane transducers of both positive and negative signals that regulate cell growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01457-20 DMN

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Chemical Synthesis of Radioactive Sphingolipids

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Andrew E. Gal, Ph.D., Chief, Neurochemical Meth.  
OTHERS: Patricia J. Voorstad, Chemist

DMN NINCDS  
DMN NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Neurochemical Methodology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Sphingolipids containing radioactive isotopes were synthesized and used for metabolic studies and as diagnostic tools in sphingolipidoses.  $^{14}\text{C}$  and  $^3\text{H}$  labels were introduced by synthetic and semi-synthetic techniques, gas exposure, and a new approach: functional group exchange. These techniques were used for the syntheses of radioactive enantiomorphous derivatives of sphingolipids. These products are not metabolizable. Experimentation with these in animals creates "animal models" for metabolic diseases and opens new areas for biomedical studies.

12 DMN/IRP

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01808-17 DMN

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Glycoproteins of Myelin in Development and Disease

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. H. Quarles, Ph.D. Section Chief DMNB, NINCDS  
 Others: A. Noronha, Ph.D. Visiting Fellow DMNB, NINCDS  
 A. Ilyas, Ph.D. Visiting Fellow DMNB, NINCDS  
 D. O'Shannessey, Ph.D. Visiting Fellow DMNB, NINCDS  
 K. Yanagisawa, M.D. Visiting Fellow DMNB, NINCDS  
 Hugh Willison, M.D. Visiting Fellow DMNB, NINCDS  
 Johanna Moller, M.D. Visiting Fellow DMNB, NINCDS  
 Roscoe O. Brady, M.D. Branch Chief DMNB, NINCDS

## COOPERATING UNITS (if any)

E.K. Shriver Center for Mental Retardation, Waltham, MA; Department of Neurology, Johns Hopkins Univ., Balto., MD; Laboratory of Molecular Genetics, NINCDS; Neuroimmunology Branch, NINCDS; Infectious Diseases Branch, NINCDS.

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Section on Myelin and Brain Development

## INSTITUTE AND LOCATION

Park Building, Rm. 425, NINCDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

8.9

## PROFESSIONAL:

6.8

## OTHER:

2.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The myelin-associated glycoprotein (MAG) is localized in the periaxonal membranes of PNS and CNS myelin sheaths where it appears to be involved in glia-axon interactions. The amount of MAG in the brains of myelin deficient rats with a severe hypomyelination of the CNS due to a sex linked mutation is reduced to 2% of the control level, and proteolipid protein (PLP) is not detected suggesting that the gene for PLP that is on the X-chromosome may be affected directly. In multiple sclerosis, MAG is reduced more than PLP or myelin basic protein in periplaque areas, and in many lesions much of the MAG is in the form of its proteolytic derivative, dMAG. Glycoconjugates sharing carbohydrate epitopes with MAG and reacting with various monoclonal antibodies such as human IgM paraproteins associated with neuropathy and HNK-1 were studied further. Newly identified 19-26K dalton glycoproteins of PNS myelin that react with these antibodies have been purified and partially characterized. Testing the antigenicity of chemically modified derivatives of the major PNS-specific glycolipid reacting with these antibodies, 3-sulfate glucuronyl paragloboside, revealed idiotypic heterogeneity among the human anti-MAG IgM paraproteins. The cat is a suitable experimental animal for investigating the pathogenicity of human anti-MAG IgM paraproteins, since it expresses each of the PNS antigens reacting with the antibodies, but experiments in cats have not provided strong evidence to suggest that the human antibodies cause the neuropathy. A high proportion of patients with IgM gammopathy and neuropathy in which the IgM does not react with MAG have paraproteins that react with other acidic glycolipids, indicating that glycolipid antigens are common in neuropathy associated with IgM gammopathy. Other antigens that have been identified include GM2, GM1 and GD3, GD1B and GT1B gangliosides. Some patients with Guillain-Barre syndrome also have high titers of antibodies reacting with acidic glycolipid antigens.

13 DMN/IRP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 NS 02162-12 DMN</b>
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Synthesis of Compounds Analogous to Glycolipids</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div> <b>PI: Andrew E. Gal, Ph.D., Chief, Neurochemical Methodology Section</b>  <b>OTHER: Patricia J. Voorstad, Chemist, Neurochemical Methodology Section</b> </div> <div style="text-align: right;"> <b>DMN, NINCDS</b>  <b>DMN, NINCDS</b> </div> </div>		
COOPERATING UNITS (if any) <div style="text-align: center; margin-top: 10px;"> <b>None</b> </div>		
LAB/BRANCH <div style="text-align: center; margin-top: 10px;"> <b>Developmental and Metabolic Neurology Branch</b> </div>		
SECTION <div style="text-align: center; margin-top: 10px;"> <b>Neurochemical Methodology Section</b> </div>		
INSTITUTE AND LOCATION <div style="text-align: center; margin-top: 10px;"> <b>NINCDS, NIH, Bethesda, MD. 20892</b> </div>		
TOTAL MAN-YEARS: <div style="text-align: center; margin-top: 10px;"> <b>1.4</b> </div>	PROFESSIONAL: <div style="text-align: center; margin-top: 10px;"> <b>0.7</b> </div>	OTHER: <div style="text-align: center; margin-top: 10px;"> <b>0.7</b> </div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <div style="margin-top: 20px;"> <p>Work was continued on the syntheses of glycolipid analogues of sphingolipids that yield a chromogenic moiety on enzymatic hydrolysis. These compounds are used for the diagnosis and studies of <u>Niemann-Pick, Gaucher's and Krabbe's disease</u>.</p> <p>Conduritol B epoxide, a saccharide that strongly inhibits <math>\beta</math>-glucosidases, was synthesized by a method developed by this section that provides the product in greater yield than previously available and permits the preparation of this compound containing a tracer with extraordinarily high specific radioactivity. Administration of conduritol B-epoxide to animals produces a syndrome that resembles <u>Gaucher's disease</u> in humans by inhibiting the enzyme glucocerebrosidase. Radioactive conduritol B-epoxide was also synthesized by a novel method which allows the production of large quantities in highly active form of this product. It reacts with the active site of glucocerebrosidase isolated from normal human tissues and from patients with Gaucher's disease. This use of the radioactive conduritol <math>\beta</math>-epoxide will materially accelerate the identification of the <u>amino acid substitutions (or deletions)</u> that occur in the glucocerebrosidase molecule in patients with <u>Gaucher's disease</u>. Also fluorescent and cytotoxic substrates were prepared which can be used for the separation of affected and normal cells.</p> </div>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02163-12 DMN

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Analytical Methods for the Use of Research of Sphingolipidoses

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Andrew E. Gal, Chief, Neurochemical Methodology Section DMN, NINCDS  
OTHER: Patricia J. Voorstad, Chemist, Neurochemical Methodology DMN, NINCDS  
Section

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Neurochemical Methodology Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20892

## TOTAL MAN-YEARS:

0.2

## PROFESSIONAL:

0.1

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

New analytical techniques were developed and used in enzymatic research and in clinical investigations of lipidoses. The lipid content in human tissues, the diagnosis of lipid storage diseases by gas, thin-layer chromatography and other techniques were studied at the microgram level. The techniques we developed previously were improved, modified and used in connection with ongoing projects related to lipidoses in our laboratories and also as joint projects with outside groups. Numerous analytical studies were undertaken by using these techniques.

15 DMN/IRP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02366-08 DMN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Hormone-Responsive Adenylate Cyclase		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: P. H. Fishman, Ph.D., Chief, Membrane Biochem. Section, DMN, NINCDS OTHERS: R. V. Rebois, Ph.D., Senior Staff Fellow, DMN, NINCDS S. Kassiss, Ph.D., Visiting Associate, DMN, NINCDS A. Sidhu, Ph.D., Visiting Fellow, DMN, NINCDS R. M. Bradley, B.S., Chemist, DMN, NINCDS M. A. Sullivan, B.S., Biologist, DMN, NINCDS M. Olasmaa, Guest Researcher, Upsala University		
COOPERATING UNITS (if any) Laboratory of Clinical Science, NIMH		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Membrane Biochemistry Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	3.7	2.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) 1. Exposure of mammalian cells to isoproterenol resulted in a rapid loss of agonist-stimulated <u>adenylate cyclase</u> activity. <u>Desensitization</u> was accompanied by a sequestration of $\beta$ -adrenergic receptors into a lighter density membrane fraction inaccessible to hydrophilic antagonists. When the cells were washed free of agonist, resensitization and reappearance of the sequestered receptors occurred. Pretreatment of the cells with concanavalin A blocked sequestration but not desensitization. Using a <u>membrane fusion</u> technique to transfer the receptors to a foreign adenylate cyclase, we were able to show that a reduction in receptor function occurred during desensitization and in the absence of sequestration. Upon resensitization, receptor function was recovered. Thus, the key event in agonist-mediated desensitization is a reduction in receptor function. 2. Exposure of rat striatal membranes to N-ethylmaleimide (NEM) caused a loss of <u>D-1 dopamine receptors</u> . <u>D-1 specific agonists or antagonists</u> , respectively, fully and partially protected the receptors from inactivation by NEM. Upon transfer of the receptors to a foreign adenylate cyclase by membrane fusion, agonist- but not antagonist-protected D-1 receptors remained functional as measured by agonist stimulation of the cyclase. We have now succeeded in solubilizing the D-1 receptor and are using the selective protection by agonist as a means to purify the receptor. 3. Deglycosylated <u>human chorionic gonadotropin</u> (DG-hCG) is a potent antagonist of hCG. When DG-hCG is bound to hCG-receptors on MLTC-1 cells, addition of anti-hCG reverses the antagonism and results in stimulation of adenylate cyclase. Rabbit antibodies raised to DG-hCG cross-reacted with hCG. When the antiserum was purified on an hCG-agarose affinity column, the nonabsorbed fraction only reacted with DG-hCG and did not reverse its antagonism. In contrast, the absorbed antibodies reacted preferentially with the $\beta$ -subunit of hCG and DG-hCG, reversed the antagonism of DG-hCG and this reversal was blocked by prior incubation of the antibodies with the $\beta$ - but not the $\alpha$ -subunits. Thus, epitopes on the $\beta$ -subunit may be important for determining whether the hormone is an agonist or antagonist.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02435-07 DMN

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Mechanism of Pathogenesis of the Mucopolysaccharidoses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: George Constantopoulos, Ph.D., Research Biochemist, DMN, NINCDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Enzymology and Genetics

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20892

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.5

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mucopolysaccharidoses (MPS) are a group of hereditary diseases characterized by defective metabolism of glycosaminoglycans (GAGs). The disorders are usually associated with severe dysfunction of the nervous system as well as of liver, spleen, heart, bone, and other tissues. Objective of this project is the study of mechanism of pathogenesis of these diseases with emphasis on brain involvement and mental retardation. We are using a comparative approach. For this purpose we study the changes, in GAGs, sphingolipids, and pertinent lysosomal enzymes in tissues of patients with various types of MPS and we make correlation in terms of clinical and ultrastructural findings. Our laboratory contributed significantly in understanding the chemical pathology and in particular the neurochemistry of MPS IH, MPS IS, MPS II, MPS III A and MPS III B. To complement the studies with human subjects, a drug (suramin) induced animal model of MPS has been developed and a canine model, (natural), of MPS I ( $\alpha$ -L-iduronidase deficiency), has been fully characterized. In an attempt to reverse the progressive deterioration caused by this incurable metabolic disorder, five dogs with MPS I were transplanted with marrow from normal or heterozygous littermates. Transplanted bone marrow provides the affected dogs with self-renewing source of cells that produce the enzyme needed to complete the metabolic process. One year after transplantation the results are very encouraging for this neurodegenerative metabolic disorder because we found marked corrective changes within the central nervous system of the dogs.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02453-06 DMN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gaucher's Disease: Biochemical and Clinical Studies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Roscoe O. Brady, M.D., Chief, DMNB, IRP, NINCDS OTHERS: Norman Barton, M.D., Ph.D, John Fink, M.D. Bing Wen-Soong, M.D., Warren Cohen, M.D., Ph.D., Gregory Zirzow, Mark Gariel, Drs. H. Mankin and S. Doppelt, MGH, Boston, MA.; Dr. J. Tager, Univ. of Amsterdam; Dr. Arnold Reuser, Erasmus Univ., The Netherlands		
COOPERATING UNITS (if any) Dept. of Orthopaedic Surgery, Massachusetts General Hospital, Boston, MA; Dept. of Biochem., Univ. of Amsterdam, The Netherlands; Dept. of Genetics, Erasmus Univ., The Netherlands; Children's Hospital, Washington, D.C.		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Clinical Investigations & Therapeutics Section, Enzymology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">4.5</div>	PROFESSIONAL: <div style="text-align: center;">3.5</div>	OTHER: <div style="text-align: center;">1.0</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           Conventional or novel therapy for Gaucher's disease depends upon broad clinical and basic scientific knowledge of the disorder. Many patients have been studied and important complications identified. Diagnosis of <u>different phenotypes</u> using a monoclonal antibody permits identification of neurologically affected cases presymptomatically. A disorder metabolism affecting calcium homeostatis has been described and regimens of <u>vitamin D</u> and calcium supplementation are being evaluated. Basic research work on glucocerebrosidase has generated a variety of projects which address the <u>biochemistry, cell biology, and molecular genetics</u> of the enzyme as a part of more far-reaching studies. Glucocerebrosidase serves as a model for these studies of lysosomal enzymes and proteins. The results of this coordinated approach have revealed the <u>structure, biosynthesis, rates of synthesis and degradation, lysosomal routing, lectin binding, and cellular uptake</u> of the enzyme. Alterations of some of these processes have been described for several mutations of the gene resulting in different phenotypes of the disease. This information provides data from which the approach of <u>enzyme replacement</u> is perfected. A <u>clinical trial</u> incorporating these advances is currently underway. Other projects have resulted in the isolation, expression, and transfer of the <u>gene</u> for glucocerebrosidase leading to the consideration of <u>gene transfer</u> for Gaucher's disease.         </p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02529-05 DMN

PERIOD COVERED

October 1, 1985 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Enzymes that Inactivate Neurotoxic Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. O. Brady, M.D., Chief, DMN	NINCDS
OTHERS:	J. M. Poston, Ph.D.	NHLBI
	A. E. Gal, Ph.D.	DMN
		NINCDS

COOPERATING UNITS (if any)

Laboratory of Biochemistry, NHLBI

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Enzymology and Genetics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS-02619-03 DMN
PERIOD COVERED      October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)      Oxidative Metabolism in Patients with Inherited Neurological Diseases and in Mycoplasmas.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  PI: George Constantopoulos, Ph.D., Research Biochemist,      DMN, NINCDS		
COOPERATING UNITS (if any)  Institute for Medical Research, Camden, New Jersey Surgical Neurology Branch, NINCDS		
LAB/BRANCH      Developmental and Metabolic Neurology Branch		
SECTION      Enzymology and Genetics		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD.      20892		
TOTAL MAN-YEARS:      0.8	PROFESSIONAL:      0.3	OTHER:      0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided )  <p>           An increasing amount of evidence points to a possible defect in <u>oxidative metabolism</u> in patients with certain <u>inherited neurological disorders</u>. Thus, a defect in the <u>pyruvate oxidation system</u> has been shown in some patients with lactic acidemia and diffuse neurologic disease, of the <u>mitochondrial malic enzyme</u> in patients with <u>Friedreich's ataxia</u>, and a partial deficiency of <u>glutamate dehydrogenase</u> in some patients with <u>olivopontocerebellar degeneration</u>. However, there is much controversy about the exact enzymatic defect(s). The objective of this project is the elucidation of the defect in some of these patients or in skin <u>fibroblasts</u> derived from such patients. For this purpose we are assaying a number of <u>mitochondrial and non-mitochondrial enzymes</u> in <u>fibroblasts or leukocytes</u> and we have initiated <u>electron microscopic studies</u> of the <u>mitochondria</u>. We became interested in the oxidative metabolism of <u>mycoplasmas</u> because <u>mycoplasma contamination of fibroblast cultures</u> interfered with the assay of <u>pyruvate dehydrogenase complex</u> in these cells. The oxidative metabolism of mycoplasmas is poorly understood. Hopefully, the elucidation of the defect in these diseases will help in the diagnosis and therapeutic intervention in the patients. Knowledge of the physiology of mycoplasmas may help in understanding the pathogenicity of these organisms.         </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02648-02 DMN

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Modification of Human Glioma Cells In Vitro

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: George Constantopoulos, Ph.D., Research Chemist, DMN, NINCDS  
OTHERS: Roscoe O. Brady, M.D., Chief DMN, NINCDS  
Paul L. Kornblith, M.D., Chief SNB, NINCDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, NINCDS

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Enzymology and Genetics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Shedding of various products from the cell surface of cancer cells may have an important role in loss of adhesion, metastasis, generation of immune-blocking factors and other pathophysiologic aspects of cancer (Black PH, N. Engl. J. Med. 303: 1315, 1980). Human glioma cells in tissue culture produce and shed in the media much greater amounts of glycosaminoglycans (GAGs) than normal glial cells (Glimelius et al Biochem. J., 172: 443, 1978). Glycosaminoglycans are polyanionic compounds, usually bound covalently to a protein core. They are a prominent component of the cell surface and are implicated in cell-cell interaction. There is also evidence for existence of hyaluronidase-sensitive protective coats on some neoplastic cell lines including gliomas, and it has been suggested that the protective GAG coat may impede the immune response and the efficacy of chemotherapy. Our objective was to modify or prevent the synthesis and shedding of GAGs in human glioma cells in culture, and possibly to render them more susceptible to chemotherapy and more immunologically responsive. For this purpose we use singly or in combination, the differentiating agents dimethyl sulfoxide (DMSO), sodium butyrate, retinoic acid, and dexamethasone. These agents are known to affect the synthesis of GAGs in other systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02657-02 DMN
PERIOD COVERED      October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular and Genetic Studies of Niemann-Pick Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Norman Barton, M.D., Ph.D. OTHER: Katherine Oliver, Biologist		CITS, DMN, NINCDS CITS, DMN, NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH      Developmental and Metabolic Neurology Branch		
SECTION      Clinical Investigations & Therapeutics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD. 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Niemann-Pick disease is a progressively debilitating, neurogenetic disorder</u> which is characterized biochemically by the accumulation of sphingomyelin in several tissues and organs in conjunction with deficiency of the lysosomal hydrolase, <u>sphingomyelinase</u>. Detailed description of various phenotypes in terms of cellular pathochemistry and molecular genetics has not been accomplished to date. A major obstacle in this area has been the consistent absence of reproducible techniques for the isolation of <u>homogeneous</u> preparations of sphingomyelinase. Employing novel detergent and chromatography systems, we have purified sphingomyelinase to homogeneity. The purified enzyme migrates with an <u>apparent molecular weight of 67,000 daltons</u> in SDS-polyacrylamide gels under both reducing and nonreducing conditions. Kinetic analyses and determinations of the primary protein structure and carbohydrate composition are in progress as are efforts to develop and characterize monoclonal and polyclonal antibodies to the purified enzyme. Availability of well characterized antibodies will allow us to proceed to cloning of the gene for sphingomyelinase. Characterization of the phenotypes of Niemann-Pick disease in terms of <u>protein polymorphisms</u> and <u>specific mutations</u> at the DNA level will be undertaken.         </p>		

22 DMN/IRP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02658-02 DMN

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sites of Carbohydrate Attachment in Lysosomal Enzymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brian M. Martin, Ph.D., Visiting Scientist, Molecular & Medical Genetics Section,  
DMNB, IRP, NINCDS

Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;  
Denise Merkle-Lehman, and Gary Murray, Ph.D., Clinical Investigations  
& Therapeutics Section, DMNB, NINCDS;  
Edward I. Ginns, M.D., Ph.D. and June Mayor, Molecular and Medical  
Genetics Section, DMNB, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular and Medical Genetics Sect./Clin. Investigations & Therapeutics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project discontinued due to transfer of principal investigator to NIMH.

23 DMN/IRP

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01NS02659-02 DMN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Protein Structure of Lysosomal Enzymes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Brian Martin, Ph.D., Visiting Scientist, Molecular & Medical Genetics Section, DMNB, IRP, NINCDS Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS Denise Merkle-Lehman, Gary Murray, Ph.D. and Norman Barton, M.D., Ph.D., Clin. Investigations & Therapeutics Section, DMNB, NINCDS; Edward I. Ginns, M.D., Ph.D., Molecular and Medical Genetics Section, DMNB, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Molecular & Medical Genetics Sect./Clin. Investigations & Therapeutics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.4	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  Project discontinued due to transfer of principal investigator to NIMH.		

## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02660-02 DMN

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of the Active-Site of Glucocerebrosidase

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brian Martin, Ph.D., Visiting Scientist, Molecular & Medical Genetics Section,  
DMNB, IRP, NINCDSOthers: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;  
Denise Merkle-Lehman, and Gary Murray, Ph.D., Clinical Investigations  
and Therapeutics Section, DMNB, NINCDS;  
Edward I. Ginns, M.D., Ph.D., Molecular and Medical Genetics Section,  
DMNB, NINCDS

Andrew Gal, Ph.D., DMNB, NINCDS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Molecular and Medical Genetics Sect./Clinical Invest. &amp; Therapeutics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.4

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project discontinued due to transfer of principal investigator to NIMH.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01NS02661-02 DMN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cell Biology and Biochemistry of Lysosomal Proteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Gary Murray, Ph.D., Visiting Associate, CITS, DMNB, NINCDS Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS; Edward I. Ginns, M.D., Ph.D. & Brian Martin, Ph.D., MMGS, DMNB, NINCDS; Susan Sorrell, Lori Hampton, Carol Moore, Lynn DeVaughn, Donna Huang, Mark Garfield, Gregory Zirzow & Pijush Das, Ph.D., CITS, DMNB, NINCDS; Ann Erickson, Ph.D., Rockefeller University; Joseph Tager, Ph.D., Univ. of Amsterdam; and Arnold Reuser, Ph.D., Erasmus University.		
COOPERATING UNITS (if any) University of Amsterdam, Department of Biochemistry Erasmus University, Department of Genetics Rockefeller University		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Clinical Investigations and Therapeutics Sect./Molecular and Medical Genetics Sect.		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 6.0	PROFESSIONAL: 5.7	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Project terminated due to departure of principal investigator from DMNB.		



## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02662-02 DMN

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Engineering of Human Lysosomal Enzymes: Studies of Enzyme Replacement

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gary J. Murray, Ph.D., Clin. Investigations &amp; Therapeutics Section, DMNB, NINCDS

Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;  
Brian Martin, Ph.D. & Edward Ginns, M.D., Ph.D., MMGS, DMNB;  
Mark Garfield, Susan Sorrell, Carol Moore, Gregory Zirzow and  
Henry O'Connell, CITS, DMNB, NINCDS.

## COOPERATING UNITS (if any)

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Clinical Investigations &amp; Therapeutics Sect./Molecular &amp; Medical Genetics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

2.5

## PROFESSIONAL:

2.3

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project terminated due to departure of principal investigator from DMNB.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01NS02663-02 DMN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Organization of Human Glucocerebrosidase Gene		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Prabhakara V. Choudary, MD., Sen. Staff Fellow, MMGS, DMNB, NINCDS Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS; Edward I. Ginns, M.D., Ph.D., Brian Martin, Ph.D., Barbara Stubblefield, Suzanne Winfield, Nancy Miller, Ph.D., Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca, and Carl Lauter, MMGS, DMNB, NINCDS; Gary Murray, Ph.D., CITS, DMNB, NINCDS. Mia Horowitz, Ph.D., Weizmann Inst. of Science, Rehovot, Israel		
COOPERATING UNITS (if any) Weizmann Institute, Rehovot, Israel; Genex Corporation, Gaithersburg, MD		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Molecular and Medical Genetics Sect./Clinical Invest. & Therapeutics Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.5	2.4	0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  Project terminated due to departure of principal investigator.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02664-02 DMN

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Neurogenetic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Norman Barton, M.D., Ph.D.

OTHERS: Roscoe O. Brady, M.D., John Fink, M.D., Bing-Wen Soong, M.D., and Cynthia West, Warren Cohen, M.D., Joseph Tager, Ph.D. and Andre Schram, Ph.D., Univ. of Amsterdam; Frank King, M.D., & Hamal Ishak, M.D., Ph.D., A.F. Inst. of Pathology; Henry Mankin, M.D., & Samuel Doppelt, M.D., MGH, Boston, MA; David Ullman, Ph.D., Veterans Hospital, M.A.

## COOPERATING UNITS (if any)

Interinstitute Medical Genetics Program, C.C., NIH; Human Genetics Branch, NICHD; Lab. of Biochem., Univ. of Amsterdam, The Netherlands; Massachusetts Gen. Hosp.; Armed Forces Inst. of Pathol.; Veterans Hosp., GRECC, Waltham, MA.

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Clinical Investigations &amp; Therapeutics Section/Enzymology and Genetics Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20892

## TOTAL MAN-YEARS:

7.0

## PROFESSIONAL:

5.5

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The clinical study of neurogenetic diseases provides the context in which the goals of improved diagnosis and potential treatment modalities are identified. Several new phenotypes have been recognized including a number of cases of deficiency of hexosaminidase A presenting as motor neuron disease; glycerol kinase deficiency presenting as acidemia and stupor without mental retardation; and bipterin deficiency presenting as familial dystonia. A number of rare phenotypes have also been identified including Tay-Sachs disease in a young non-Jewish child, 2 cases of juvenile Krabbe's disease, an unusual presentation of multiple sulfatase deficiency, an unusual case of San Filippo A disease, a mild variant of Morquio's syndrome, a case of arylsulfatase activator deficiency, a case of Menke's disease in a female infant, and several cases of acute neuronopathic Gaucher's disease. A large number of typical neurogenetic diseases have been confirmed by studies performed in this protocol. Studies of chorionic villus samples (CVS) have allowed the prenatal diagnosis of a number of lysosomal storage diseases. We have developed an accurate method for biochemically distinguishing phenotypes pre-symptomatically. Finally, a clinical trial of enzyme replacement is being conducted in Gaucher's disease. The application of gene transfer to human disease is under consideration.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01NS02665-02 DMN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Mapping Functional Domains of Lysosomal Enzymes</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Edward I. Ginns, M.D., Ph.D., Mol. & Med. Genetics Section, DMNB, NINCDS Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS; Prabhakara Choudary, Ph.D., Barbara Stubblefield, Suzanne Winfield, Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca, and Brian Martin, Ph.D. and Carl Lauter, MMGS, DMNB, NINCDS; Gary Murray, Ph.D., CITTS, DMNB, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH <u>Developmental and Metabolic Neurology Branch</u>		
SECTION <u>Molecular and Medical Genetics Sect./Clinical Invest. &amp; Therapeutics Section</u>		
INSTITUTE AND LOCATION <u>NINCDS, NIH, Bethesda, MD 20892</u>		
TOTAL MAN-YEARS: <u>2.0</u>	PROFESSIONAL: <u>1.9</u>	OTHER: <u>0.1</u>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p style="text-align: center;">Project discontinued due to transfer of principal investigator to NIMH.</p>		

30 DMN/IRP

## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02666-02 DMN

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Application of Gene Transfer to the Correction of Inherited Enzyme Deficiencies

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Nancy Miller, Ph.D., Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca,  
and Brian Martin, Ph.D., & Carl Lauter, MMGS, DMNB, NINCDS;  
Gary Murray, Ph.D., CITS, DMNB, NINCDS;  
Dr. Richard Mulligan & Dr. Connie Cepko, Whitehead Institute, MIT.

## COOPERATING UNITS (if any)

Massachusetts Institute of Technology, Whitehead Institute, Cambridge, MA

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Molecular and Medical Genetics Section, Clin. Investigations &amp; Therap. Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.5

## PROFESSIONAL:

2.3

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project discontinued due to transfer of principal investigator to NIMH.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01NS02681-02 DMN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Genetic Studies of the Mucopolysaccharidoses IH, IH/S and IS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Edward I. Ginns, M.D., Ph.D., Molecular & Medical Genetics Sec., DMNB, NINCDS Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS; Prabhakara V. Choudary, Ph.D., Brian Martin, Ph.D., Barbara Stubblefield, Suzanne Winfield, Nancy Miller, Ph.D., Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca, and Carl Lauter, MMGS, DMNB, NINCDS; Gary Murray, Ph.D., CITS, DMNB, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Developmental and Metabolic Neurology Branch		
SECTION Molecular and Medical Genetics Sect./Clinical Investigations & Therapeutics Sect.		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 2.0	PROFESSIONAL 1.9	OTHER 0.1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div> <input type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Project discontinued due to transfer of principal investigator to NIMH.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02682-02 DMN

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Lysosomal Disorders

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Edward I. Ginns, M.D., Ph.D., Molecular and Medical Genetics Sect., DMNB, NINCDS,  
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS  
Prabhakara V. Choudary, Ph.D., Brian Martin, Ph.D., Barbara Stubblefield,  
Suzanne Winfield, Nancy Miller, Ph.D., Shoji Tsuji, M.D., Ph.D., June  
Mayor, Mary LaMarca, and Carl Lauter, MGS, DMNB, NINCDS;  
Gary Murray, Ph.D., CITS, DMNB, NINCDS  
Drs. J. Tager & A. Schram, Dept. of Biochem., Univ. of Amsterdam

## COOPERATING UNITS (if any)

Department of Biochemistry, University of Amsterdam, The Netherlands

## LAB/BRANCH

Developmental and Metabolic Neurology Branch

## SECTION

Molecular and Medical Genetics Sect./Clinical Investigations &amp; Therapeutics Sect.

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.5

## PROFESSIONAL:

2.3

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project discontinued due to transfer of principal investigator to NIMH.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01NS02683-02 DMN
PERIOD COVERED <u>October 1, 1985 through September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Study of Eukaryotic Shuttle Vectors for Human Gene Transfer</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Prabhakara V. Choudary, Ph.D., Senior Staff Fellow, DMNB, NINCDS Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS; Edward Ginns, M.D., Ph.D., Brian Martin, Ph.D., Barbara Stubblefield, Suzanne Winfield, Nancy Miller, Ph.D., Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca and Carl Lauter, MMGS, DMNB, NINCDS; Gary Murray, Ph.D., CITS, DMNB, NINCDS; Dr. Richard Mulligan and Dr. Connie Cepko, Whitehead Institute, MIT.		
COOPERATING UNITS (if any)  Massachusetts Institute of Technology, Whitehead Institute, Cambridge, MA; Meloy Laboratories, Springfield, VA		
LAB/BRANCH <u>Developmental and Metabolic Neurology Branch</u>		
SECTION <u>Molecular and Medical Genetics Sect./Clinical Investigations &amp; Therapeutics Sect.</u>		
INSTITUTE AND LOCATION <u>NINCDS, NTH, Bethesda, MD 20892</u>		
TOTAL MAN-YEARS: <u>3.0</u>	PROFESSIONAL: <u>2.8</u>	OTHER: <u>0.2</u>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects              <input type="checkbox"/> (a1) Minors              <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  Project discontinued due to departure of principal investigator.		







ANNUAL REPORT

October 1, 1985 through September 30, 1986

Experimental Therapeutics Branch

National Institute of Neurological and  
Communicative Disorders and Stroke

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## ANNUAL REPORT

October 1, 1985 through September 30, 1986

### Experimental Therapeutics Branch

National Institute of Neurological and Communicative Disorders and Stroke

Thomas N. Chase, M.D., Chief

The Experimental Therapeutics Branch directs its investigative efforts towards the rational development of improved pharmacotherapies for disorders of the human central nervous system. A highly integrated program of fundamental and applied research seeks to define relationships between clinical signs of brain dysfunction and specific alterations in neuronal transmission; based on a detailed understanding of synaptic mechanisms and of potential sites for pharmacologic intervention, novel therapeutic approaches are developed to modify the affected system and thus improve clinical function. Branch research, at both clinical and preclinical levels, remains focused on the dopamine system and selected peptidergic pathways in relation to Parkinson's disease and Alzheimer's disease.

The Branch is currently organized into four closely integrated components: Dr. John Kebabian's Biochemical Neuropharmacology Section has carried out basic biochemical and pharmacologic studies of dopamine receptor mechanisms. Dr. Judith Walters' Physiological Neuropharmacology Section evaluates interactions between the dopamine system and other transmitter pathways within the basal ganglia. Dr. Thomas O'Donohue's Neuroendocrinology Unit has investigated peptidergic systems involved in cognitive and motor function. Dr. Thomas Chase's Pharmacology Section explores transmitter abnormalities and pharmacologic interventions in dementing and extrapyramidal disorders. Both Dr. Kebabian and Dr. O'Donohue have recently left for senior positions in the pharmaceutical industry; Dr. Masahide Munemura and Dr. Bibie Chronwall will serve as Acting Chiefs of their respective groups until the search for permanent replacements has been completed.

### Biochemical Neuropharmacology Section

During FY 85, the Section continued to focus attention upon its two areas of traditional strength, dopamine receptor pharmacology and pituitary cell biology.

The iodinated ligand ( $^{125}\text{I}$ ) SCH 23982 was used to further characterize the D-1 receptor in the caudate putamen and the substantia nigra. The ligand also binds to melanin by a mechanism unrelated to its interactions with the D-1 receptor. Because the molecule is a gamma-emitting compound, it can be used for the non-invasive imaging of melanomas.

The Section has also studied the involvement of calcium and cAMP in the process of hormone secretion from the pituitary gland. Using the 7315c tumor, the Section showed that cAMP potentiated the response to a fixed increment in cytosolic calcium. This effect was reproduced in cells which had been permeabilized by intense electrical discharges.

The coupling of the TRH receptor to phospholipase C has been evaluated. The data suggest that the coupling protein is distinct from the classically recognized  $N_s$  and  $N_i$  molecules.

The Section also completed its studies of cAMP-dependent protein phosphorylation in the intermediate lobe of the rat pituitary gland. cAMP-dependent phosphorylation was demonstrated in both homogenates and permeabilized IL cells.

## NEUROENDOCRINOLOGY UNIT

### Pharmacology and Cellular Biology of Peptidergic Neurons

The most recently identified and the major known class of neurotransmitters and hormones is comprised of peptides. The goal of the Unit has been to develop an understanding of the basic regulatory mechanisms in cells which secrete peptides, and through this understanding, develop novel pharmacotherapeutic approaches and agents for manipulating peptidergic systems. Two projects are ongoing. The first studies the regulation of biosynthesis of peptides. The primary model under investigation is the opiomelanocortin containing neuronal and endocrine system which secretes ACTH,  $\alpha$ -MSH and  $\beta$ -endorphin. These peptides are derived from a single prohormone (pro-opiomelanocortin or POMC) and influence arousal and cognitive processes through interactions with central MSH receptors and analgesia through interactions with mu and delta opioid receptors. The second investigation is focused on studies of an endogenous peptide ligand which interacts with the sigma opioid receptor.

#### 1. Regulation of biosynthesis of peptides

Biosynthesis of peptides occurs in three steps. First, the process is initiated by signal transduction between cell surface receptors and the biosynthetic mechanisms. Second, peptide prohormone and processing enzymes are synthesized. Third, the final secretory form of the peptide is generated by cleavage and modification of the prohormone by post-translational processing enzymes.

In the last four years, the Unit investigated the third biosynthetic step -- the roles and regulation of post-translational processing of POMC. It was found that post-translational processing of the POMC-derived peptides dramatically changes their biological and behavioral activity. The POMC derived peptides,  $\alpha$ -MSH and  $\beta$ -endorphin, bind to different postsynaptic receptors but have extensive interactions. In FY 84, it was found that the extent of post-translational processing of POMC was not only tissue specific but also varied in different physiological situations. For example, it was observed that there are different ratios of POMC derived peptides in different tissues and this ratio changes in physiological situations. In FY 85 and 86, the Unit has continued investigations of regulation of post-translational processing of POMC and post-translational processing enzymes. It was found that the biosynthesis of POMC and certain POMC post-translational processing enzymes is coordinately regulated and can be co-induced or co-inhibited by regulation of cell surface receptors.

In the last two years, the Unit investigated the second biosynthetic step -- the mechanism of induction of POMC biosynthesis primarily using the POMC-containing cells of the intermediate lobe of the pituitary as a model. It was found that there are temporal differences in the way a neuroendocrine system regulates peptide biosynthesis. After long-term stimulation or inhibition, the secretory cells of the intermediate lobe proliferate or die in response to the requirement for POMC secretion. After a moderate length of stimulation or inhibition, inactive cells are recruited or active cells turned off. After acute stimulation, the biosynthesis of POMC is induced by increasing the intracellular content of POMC mRNA. The increase in POMC mRNA could be due to either a decrease in degradation rate of POMC or an increase in the POMC mRNA transcription rate. In cultured corticotroph tumor cells, we have found that stimulation of corticotropin releasing factor (CRF) receptors on the cell surface induces transcription of POMC mRNA within minutes. It therefore appears that the primary site of POMC biosynthesis regulation is at the transcriptional level. In addition, it was found that there is a biphasic induction of POMC biosynthesis. The first phase of induction of POMC mRNA occurs in the first two hours and the second phase occurs at about 12 hours. It was found that both of these peaks are due to transcriptional regulation. The Unit also found that thymosin, an immune peptide also regulates the secretion and biosynthesis of POMC peptides in the pituitary.

In FY 85, the Unit began studies on the mechanism of signal transduction between cell surface receptors and the biosynthetic process. The induction of POMC gene transcription was found to be a cAMP-protein kinase A dependent mechanism in both corticotrophs and melanotrophs. It was also found that the diacylglycerol-protein kinase C system is involved in regulating biosynthesis in corticotrophs. A systematic study of protein kinase A and protein kinase C substrates for phosphorylation was begun to determine the putative third messengers involved in transmitting information from cell surface receptors to the nucleus. In FY 86, a number of phosphoproteins associated with induction of POMC transcription and POMC peptide secretion were identified.

## 2. An endogenous peptide ligand for the sigma opioid receptor

$\beta$ -endorphin, enkephalin and dynorphin have been identified as endogenous peptide ligands for the mu, delta and kappa opioid receptors. These receptors appear to be involved in the analgesic and reward processes of opioids. The sigma opioid receptor, according to the original classification, mediates the psychotomimetic properties of certain opiates including phencyclidine (PCP) and SKF 10,047. In FY 83, the Unit identified a peptide,  $\alpha$ -endopsychosin, in the central nervous system which binds to the phencyclidine receptor. In addition, this peptide shares behavioral and electrophysiological activities of PCP. The peptide has been purified to homogeneity and was found in highest concentrations in cerebral cortex and hippocampus.

Ligands used to identify the sigma opioid receptor include phencyclidine, SKF 10,047 and dextroalprazolam. All of these compounds produce psychotomimetic actions in rats and humans and were thought to do so by interactions with a single receptors. In FY 85, we found that the sigma opioid receptor is not a single site but is composed of at least two, and probably three different binding sites. The highest density of all the subtypes of receptors is located

in the cerebral cortex and hippocampus. These sites which contain the highest densities of the PCP-like peptide. The localization of receptors and peptide in these sites is consistent with both the psychotomimetic and cognitive effects of these compounds. It is now clear that there is a PCP preferring receptor and an SKF 10,047 or sigma preferring receptor.

In FY 86, the Unit identified  $\beta$ -endopsychosin, an endogenous ligand for the sigma opioid receptor. The peptide is structurally distinct from  $\alpha$ -endopsychosin is a novel peptide and has a distinct pharmacology from PCP. Peptide analogs of this peptide have been synthesized and a small biologically active sequence has been identified. This sequence is being used for further studies to design new agonists and antagonists for the sigma receptor.

In FY 84, the Unit developed Metaphit, the first compound that can be used as a PCP/sigma opioid antagonist. Metaphit is a PCP receptor acylating agent. In FY 85, the mechanism of action of Metaphit was demonstrated. In FY 86, a number of analogs of Metaphit were synthesized and are currently being tested for activity. A fluorinated form of PCP was also synthesized and found to be active. This compound will be used for analysis of PCP binding sites in primates using PET scanning.

#### Physiological Neuropharmacology Section

This project involves investigation of the role of specific neurotransmitters in regulating neuronal activity in extrapyramidal systems. Current focus is on the nigrostriatal dopamine system which plays an important role in information processing in the basal ganglia. The function of these tonically active catecholamine neurons appears to involve regulation of gain and modulation of the effectiveness with which other neurotransmitter systems transmit information. These cells do not appear involved with transmission of specific phasic messages. Dysfunction of this neuronal system has been implicated in the etiology of many neurological diseases, including Parkinson's disease, tardive dyskinesia, schizophrenia and Huntington's Chorea.

In the past year, we have continued using neurophysiological techniques to explore the mechanisms underlying dopamine's critical role in the basal ganglia and the potential for modulating dopamine function with drugs. The focus of our studies has been an examination of three current problems relating to the different dopamine receptor subtypes:

- 1) What is the role of the D-1 dopamine receptor in basal ganglia function?

For many years, it has been assumed that the dopamine receptor responsible for mediating the classic effects of dopamine agonists on behavior was the D-2 dopamine receptor. This idea was based on the observation that the relatively selective D-2 antagonists could block the effects of dopamine and dopamine agonists. Because of the lack of selective drugs and appropriate techniques for probing the function of the D-1 receptor, the significance of this receptor in the normal or diseased brain has been a long standing mystery. Our studies on dopamine agonist effects on the activity of neurons in the substantia nigra pars reticulata and the globus pallidus, the main basal ganglia output nuclei,



provided the surprising observations that dopamine agonists selective for the D-2 dopamine receptor could not mimic the effects of the nonselective agonists. This observation together with our finding that a putative D-1 antagonist could block some of the neurophysiological effects of the nonselective agonists in these two regions led to the hypothesis that the effects of dopamine and dopamine agonists on basal ganglia mediated processes might require simultaneous stimulation of both D-1 and D-2 receptor subtypes. We found that the effect of D-2 agonists on the activity of the basal ganglia output neurons were very significantly potentiated by pretreatment or coadministration of a selective D-1 dopamine receptor agonist which alone induced only small variable effects. Moreover, the inactive enantiomer of the D-1 agonist and the D-1 antagonist had no significant effect; these findings support the selectivity of the active form of these drugs for the D-1 receptor. Finally, studies in rats pretreated with alpha-methyl-para-tyrosine to block dopamine synthesis indicated that much of the effect of the D-2 agonist given alone appears dependent on endogenous dopamine providing some D-1 receptor stimulation. Consistent results have recently been obtained in behavioral studies carried out in the Therapeutics Section of the ETB. These studies have led to a fundamentally new and exciting concept about the relative roles of the two receptor subtypes: they indicate that D-1 and D-2 receptors interact synergistically to affect the activity of the striatal output cells and demonstrate the apparent necessity for both receptors to be simultaneously stimulated for the induction of processes previously thought independently mediated by the D-2 receptor. In addition, they have potentially important implications for the use of dopamine agonists and antagonists in treatment of neurological disease which are currently being explored by ETB clinicians.

- 2) Are the dopamine autoreceptors on the dopamine cell bodies and terminals different from the postsynaptic D-2 receptors?

Recently, much interest has focused on the question of whether the autoreceptors may constitute a distinct subset of receptors which could be selectively stimulated by a highly specific agonist. A drug selective for the dopamine autoreceptors might have some therapeutic advantages in the treatment of disorders like tardive dyskinesia, schizophrenia and Parkinsonism. Our previous studies had shown that one putative dopamine autoreceptor agonist, the (-) isomer of the drug 3-PPP, can act as a relatively weak agonist or partial agonist at dopamine autoreceptors but more like a dopamine antagonist at postsynaptic sites. Most recently we have used neurophysiological techniques to define the properties of two new drugs with putative selectivity for dopamine autoreceptors. Our work has shown that these drugs have properties which will make them useful for exploring the therapeutic potential of this family of agents and the theoretical basis of their mechanism of action. The two drugs, BHT 920 and EMD 38362, proved to have distinctive pharmacological profiles. Both of these drugs were found to be more effective agonists at dopamine autoreceptors than at postsynaptic dopamine receptors and both are more potent and efficacious at the autoreceptor site than (-)-3PPP. However, like (-)-3-PPP, EMD 38362's apparent selectivity for autoreceptors appears due to a partial agonist/antagonist effect at postsynaptic dopamine receptors. Thus, EMD 38362 is a drug in the same category as (-)-3-PPP, but with a greater efficacy at the autoreceptor site. BHT 920's profile is different; it does not appear to act as an antagonist at the postsynaptic receptor so has a spectrum

of activity which distinguishes it from drugs like (-)-3-PPP, EMD 23448 and EMD 38362. It simply appears to be less potent at the postsynaptic site; its weaker potency at inducing postsynaptic effects, as compared with drugs like apomorphine which are equipotent at the autoreceptor, is not compensated for by coadministration of a selective D-1 agonist nor due to significant postsynaptic partial agonist properties. Moreover, EMD 38362 has even less behavioral effect than apparent efficacy at altering other parameters involving postsynaptic dopamine receptor stimulation; its inability to produce postsynaptic behavioral effects contrasts with its ability to induce some changes in pallidal activity and, as shown by others, striatal acetylcholine release. Studies exploring the implications of these results with regard to the relative properties of the dopamine autoreceptors and the postsynaptic dopamine receptors are ongoing. Clinical trials exploring the therapeutic potential of these agents are planned as well.

- 3) How does denervation alter the consequences of dopamine receptor stimulation and affect the relative roles of the two receptor subtypes in the basal ganglia?

In recent years, research aimed at improving drug therapy for Parkinsonism has addressed the use of dopamine agonists to stimulate postsynaptic dopamine receptors in brain regions innervated by substantia nigra pars compacta dopamine neurons. As dopamine neuron loss becomes severe, the utility of such drugs in place of, or in addition to, L-dopa has shown therapeutic promise. Recently available drugs with selectivity for either the D-1 or the D-2 dopamine receptor subtype have permitted closer evaluation of early hypotheses regarding the mechanism of action of dopamine agonists which focused on their ability to stimulate D-2 receptors, while D-1 receptors were thought to be uninvolved. In fact, these studies have suggested that the consequences of stimulating the D-1 dopamine receptor subtypes are altered by chronic denervation, and that D-1 receptor stimulation induces behavioral changes like those induced by stimulation of the D-2 receptor in the denervated animals. We have confirmed this suggestion using our more sensitive neurophysiological techniques and made an additional important observation; we have found evidence for very significant interactions between the receptor subtypes in the denervated rat, an effect which had been missed in previous behavioral studies. These findings are immediately relevant to strategies for treatment of Parkinsonism and tardive dyskinesia. They suggest that a nonselective dopamine agonist would have a greater effect in a Parkinsonian patient than a dopamine agonist highly selective for one receptor subtype, especially if it were the D-2 subtype. Our most recent studies with bromocriptine further suggest that evidence that drugs exerting effects on both receptor subtypes are more effective in some respects than relatively selective agonists may already have been observed in the parkinsonian patient. Bromocriptine is a dopamine agonist used in treatment of Parkinsonism. It has little intrinsic activity for D-1 receptors and has been reported to be less effective in parkinsonian patients when administered alone than when given with L-DOPA, a drug which leads to the stimulation of both D-1 and D-2 receptors. When the neurophysiological effects of bromocriptine was examined in 6-hydroxydopamine lesioned rats, bromocriptine was found to have little effect when given alone, but when administered with a D-1 agonist at a dose which was also ineffective when given alone, potentiated effects were observed, comparable to those seen with a

nonselective dopamine agonist like apomorphine. These studies support the idea that drug effects in the 6-hydroxydopamine-lesioned rat may have significant therapeutic implications; in parkinsonian patients, as well as in 6-hydroxydopamine lesioned rats, the effectiveness of D-2 agonists in altering basal ganglia output may depend on, or be facilitated by concurrent stimulation of D-1 receptors.

## Pharmacology Section

### PARKINSON'S DISEASE

Research on Parkinson's disease continues to focus on the pathogenesis and treatment of the short term motor fluctuations which ultimately affect most of those receiving dopaminomimetic therapy. It is now clear that changes in peripheral levodopa pharmacokinetic mechanisms do not contribute to the appearance of either wearing-off or on-off phenomena. Rather, central pharmacokinetic or pharmacodynamic alterations cause the efficacy half-life of levodopa to decrease by about 35% in wearing-off and by nearly 75% in on-off patients compared with stable responders. The on-off response may thus be an exaggerated form of the wearing-off effect. Fluctuations in circulating levodopa levels are necessary but not sufficient to account for the appearance of wearing-off phenomena; central factors, possibly a critical loss of dopaminergic terminals which initially serve to buffer the antiparkinsonian response to varying circulating levodopa levels, must be contributory. Similarly, additional factors, possibly postsynaptic receptor alterations, must also be involved in the pathogenesis of on-off responses, since they do not entirely disappear with the acute stabilization of plasma levodopa levels.

Investigations during the past year continue to evaluate the therapeutic efficacy of chronic levodopa infusions in parkinsonian patients disabled by motor fluctuations. With round-the-clock, intravenous levodopa given under ambulatory conditions, both wearing-off and on-off patients evidence significantly more stable plasma drug levels and antiparkinsonian responses: Immediate reductions in motor fluctuations occur in most wearing-off patients, while those with more complex on-off phenomenon also have a decrease in response shifts, although to a lesser degree and with a slower onset rate. In an attempt to improve the safety and convenience of these infusions, studies have recently been initiated with levodopa methylester. Pharmaceutical approaches to the maintenance of constant plasma levodopa levels have also continued. The administration of an oral sustained-release levodopa-carbidopa preparation (CSR III), given every 4 to 6 hours, substantially reduced plasma levodopa variance as compared with standard levodopa-carbidopa. Motor fluctuations also diminished significantly in all wearing-off patients; on-off patients had a lesser and slower rate of motor stabilization. Clinical trials of a new sustained-release preparation with improved bioavailability have now begun.

Possible alterations in D-1 and D-2 dopamine receptor mechanisms are being explored in relation to the pathogenesis of motor response fluctuations as well as the pathophysiology of related movement disorders. In one study, D-1 dopamine receptor binding sites in rat brain were mapped by means of in vitro autoradiography with <sup>125</sup>I SCH 23982, a new and improved ligand introduced by

the Biochemical Neuropharmacology Section. We found highest concentrations of  $^{125}\text{I}$  SCH 23982 binding sites in the corpus striatum, olfactory tubercle, substantia nigra-pars reticularis and entopeduncular nucleus. Other preclinical experiments attempted to characterize functional D-1 and D-2 receptor interactions using an unusual rotational model, that is rats with unilateral striatal lesions induced by quinolinic acid. LY 171555 was observed to induce ipsilateral rotation, while SKF 38393 induced grooming and sniffing but no rotation. SKF 38393 augmented turning induced by LY 171555. LY 171555 induced rotational behavior was inhibited by alpha-methylparatyrosine or the D-1 antagonist, SCH 23390, an effect which SKF 38393 reversed. These results suggest that D-1 receptor stimulation may provide a tonic background activation which allows the phasic component of D-2 stimulation to become effective. They prompted Branch efforts to fabricate SKF 38393 for clinical studies to evaluate the effects of independent D-1 receptor stimulation and concurrent D-1 and D-2 stimulation on basal parkinsonian severity as well as on the dyskinesias and motor fluctuations which complicate levodopa treatment. Initial observations suggest that SKF 38393, either alone or in combination with levodopa, at doses comparable to those which influence central dopaminergic mechanisms in the rat, has no significant effect on motor function. Further clinical studies of this unexpected result are in progress.

Dopaminergic mechanisms have long been implicated in the pathogenesis of secondary dystonic phenomena occurring in parkinsonian patients as well as in the pathogenesis and treatment of primary dystonias. An evaluation of the ability of the direct (mainly D-2) dopamine agonist, bromocriptine, to ameliorate idiopathic dystonia has now been completed: seven patients improved substantially, 5 showed little or no change, and one had significant worsening. Improvement was sustained for over one year in 3 individuals who continued to receive bromocriptine. Although we were unable to identify any clinical features which would predict the possibility of a useful therapeutic response, our results support further studies of dopaminergic agents for the relief of dystonic phenomena. A study of the selective D-1 agonist, SKF 38393, has recently been initiated.

The possibility that alterations in non-dopaminergic systems may contribute to the pathogenesis of or provide a therapeutic approach to the response fluctuations in levodopa treated parkinsonian patients has continued to be explored, particularly in relation to the cholecystokinin (CCK-8) system. Our previous preclinical studies suggest that peripherally administered CCK-8 can modify central dopaminergic mechanisms, probably due to stimulation of CCK-8 receptors on vagal afferents. Since vagally intact parkinsonian patients evidenced no centrally mediated pharmacologic effects of a peripherally administered CCK-8 analog, this mechanism may be relatively ineffective in man. To reduce dose limiting, peripherally mediated adverse effects, these studies will now be repeated with concurrent administration of a newly developed CCK-8 receptor blocker with restricted access to the central nervous system.

The exploration of alternative approaches to the pharmacologic manipulation of cerebral CCK-8 mediated functions has remained centered on the development of drugs to inhibit inactivation of the synaptically released neuropeptide. Our previous research indicated that CCK-8 is initially cleaved intrasynaptically at the Met-3-Gly-4 bond by a membrane bound

metaloendopeptidase. Inhibitors directed against this enzyme have been synthesized which have a sequence homology with the Met-3-Gly-4 region of CCK 8 for competitive inhibition or sequence homology plus a metal chelating component or sulphydryl reducing moiety for potentially irreversible inhibition; several of these compounds have now been shown to be able to block CCK 8 degradation at low in vitro concentrations.

Emerging cerebral imaging techniques, especially those involving positron emission tomography (PET) and  $^{18}\text{F}$ -2-deoxy-2-fluoro-D-glucose (FDG) continue to be applied to the localization of dysfunctional areas in selected drug-treatment-associated (such as on-off phenomenon) and naturally occurring extrapyramidal disorders, where biochemical or histologic studies have been inconclusive. Localizing information in such cases may help direct biochemical probes towards the elucidation of critical transmitter alterations. Preliminary statistical analysis of aggregate NeuroPET image data now suggests alterations mainly in the frontal and cingulate cortex of Tourette syndrome patients and in the lenticular nuclei of patients with idiopathic dystonia. Both studies will be continued during the coming year.

#### ALZHEIMER'S DISEASE

The cause of the neuronal degeneration in Alzheimer's disease is unknown. While the search for etiologic factors continues, the application of classical transmitter pharmacology may provide an approach to the rational development of improved drugs to afford symptomatic relief. Although Alzheimer's disease has traditionally been considered a rather diffuse cortical degenerative disorder, PET-FDG studies suggest a non-uniform pattern of involvement with largest abnormalities usually in the posterior parietal-temporal region. This discovery has allowed the focusing of biochemical efforts to identify transmitter system alterations which serve as critical determinates for Alzheimer dementia.

It is now well established that cortical choline acetyltransferase (ChAT) activity and rates of acetylcholine synthesis are substantially reduced in Alzheimer's disease. Nevertheless, it is difficult to propose that the degeneration of cholinergic projections to the cerebral cortex could account for the disproportionate FDG decrement in the parietal association area: reductions in the activity of this cholinergic marker were found to be similar throughout all cortical areas examined. A recently completed clinical trial with RS-86, a selective muscarinic agonist capable of acting independently of the degenerating cholinergic neurons, revealed no consistent effect on cognitive function in Alzheimer patients at their maximal, individually tolerated dose. Although the possibility will now be explored that coadministration of an anticholinergic unable to penetrate the blood brain barrier might limit peripheral parasympathetic activation enough to allow use of a more centrally adequate RS-86 dose, taken together our results cast further doubt on the hypothesis that the cholinergic deficit, alone, accounts for Alzheimer dementia or provides a basis for effective pharmacologic intervention.

We have recently reported that spinal fluid GABA levels are characteristically reduced and others have observed a decrement in GABA

concentrations as well as in the activity of the GABA synthesizing enzyme, glutamic acid decarboxylase, in post mortem cortical tissues from Alzheimer patients. Loss of GABA neurons, especially those situated postsynaptic to cortical cholinergic projections, might contribute to the cognitive decline which occurs in these patients and account for the limited ability of cholinomimetics to ameliorate the dementia. To evaluate the possibility that pharmacologic stimulation of GABA mediated synaptic function might confer symptomatic benefit, we recently completed a clinical trial of the potent and selective GABA agonist, THIP, in patients with relatively low spinal fluid GABA levels. No relation could be found between pretreatment spinal fluid GABA levels and either baseline cognitive function or THIP induced changes in neuropsychological test performance. At maximum tolerated doses, THIP had no significant effect on cognition. Since adverse effects appeared centrally mediated, it may be reasonable to assume that THIP doses sufficient to stimulate central GABA receptors were administered and thus that GABA agonists generally are unlikely to benefit patients with Alzheimer dementia. These results also lend little support to the possibility that a loss of cortical GABA interneurons contributes significantly to the intellectual decline in Alzheimer's disease.

Evidence that a loss of cortical somatostatin neurons may relate to the dementia in Alzheimer patients continues to accumulate: we found consistently low CSF somatostatin levels in this disorder, and the degree of reduction correlated with performance on several tests of verbal and visual-spatial cognition. Spinal fluid somatostatin levels also correlated with overall cortical glucose utilization rates, due primarily to the close relation between spinal fluid values and glucose metabolism in the posterior parietal area, the region suggested by PET- FDG as first and most profoundly damaged in Alzheimer's disease. Taken together with our post-mortem results showing a somatostatin reduction in posterior parietal but not in anterior frontal areas, these data support the view that a somatostatin system deficit may be closely linked both to the parietal association cortex hypometabolism and to the symptoms which characterize Alzheimer dementia.

No drugs have yet been tested in man which selectively and potently stimulate central somatostatin-mediated function. On the other hand, a recently completed clinical trial of a somatostatin depleting drug, cysteamine, in Huntington's disease patients was designed, in part, to elucidate the role of this peptidergic system in cognitive function. Preliminary results indicate that cysteamine has no consistent effect on any of the motor or cognitive measures tested. There was, however, a significant rise in plasma growth hormone levels, indicating that at least in the hypothalamic-pituitary axis, cysteamine appropriately modified a somatostatin-mediated function. Questions left unanswered by this study will now be more directly addressed by a clinical study with a new and relatively potent somatostatin analog.

Investigations during the past year have continued to seek a more precise delineation of the dementia profile in Alzheimer patients based on the pattern of cortical dysfunction revealed by PET-FDG scans. In one approach we attempted to apply one of two nonmutually exclusive models which might account for the observed degree of behavioral heterogeneity in Alzheimer's patients. The results suggest the presence of distinct subgroups characterized by

qualitatively different profiles of cognitive impairment and corresponding patterns of cerebral hypometabolism. Thus, although Alzheimer's disease may constitute a single disease process, it does not result in a unitary dementia syndrome. Further efforts to clarify the exact pattern of intellectual decline in Alzheimer's disease will continue, especially in relation to those few patients meeting all usual clinical diagnostic criteria for Alzheimer's disease but who evidence mainly frontal lobe dysfunction.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 NS 02263-10 ET
<b>PERIOD COVERED</b> October 1, 1985 through September 30, 1986		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)</b> Biochemical and Pharmacological Studies of Dopamine Receptors		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</b> John W. Kebabian, Ph.D., Chief, Biochemical Neuropharmacology Section, ETB, NINCDS T. Agui, Visiting Fellow, Y. Furuki, Visiting Fellow, T. Yamamoto, Visiting Fellow, E. Frey, Sr. Staff Fellow, J.C. vanOene, Guest Researcher, A. Sidhu, Visiting Fellow, S. Guild, Visiting Fellow, S. Pocotte, Staff Fellow, ETB, NINCDS; T. Reisine, Staff Fellow, LCB, ADAMAHA; G. Bryant, LP, NCI, S. Larson, Nuclear Medicine, NCI; J.M. Saavedra, LCS, ADAMAHA		
<b>COOPERATING UNITS (if any)</b> Laboratory of Clinical Sciences, NIMH, ADAMAHA Department of Nuclear Medicine, CC, NIH Laboratory of Pathology, DCT, NCI		
<b>LAB/BRANCH</b> Experimental Therapeutics Branch		
<b>SECTION</b> Biochemical Neuropharmacology Section		
<b>INSTITUTE AND LOCATION</b> NINCDS, NIH, Bethesda, MD 20892		
<b>TOTAL MAN-YEARS:</b> 9.5	<b>PROFESSIONAL:</b> 9.0	<b>OTHER:</b> 0.5
<b>CHECK APPROPRIATE BOX(ES)</b> <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)</b> <p>The pharmacology of the D-1 receptor was investigated with biochemical techniques. The binding of [<sup>125</sup>I] SCH 23982 to specific binding sites in the caudate putamen and s. nigra of the rat brain was characterized. The nonspecific binding of this ligand to melanin was also investigated. This latter signal formed the basis for the noninvasive imaging of pigmented melanomas.</p> <p>The role of cAMP and calcium in the process of pituitary hormone release was investigated. cAMP elevation in the 7315c tumor cell potentiates the capacity of the cell to respond to a fixed increment in the concentration of cytosolic calcium without changing the molar potency of calcium as a stimulatory agent.</p> <p>The substrates for cAMP-dependent protein kinase endogenous to the pituitary gland were characterized in FY 86. A number of phosphate receptors were identified.</p>		

12-ET/IRP



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02578-04 ET

PERIOD COVERED

October 1, 1985 through September 30, 1986.

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology and Cellular Biology of Peptidergic Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Thomas L. O'Donohue, Ph.D., Head, Neuroendocrinology Unit, ETB, IRP, NINCDS

E. Burcher, T.N. Chase, B.M. Chronwall, P.C. Contreras, J.M. Farah, W.R. Millington, C.W. Shults, R.E. Tessel, J.R. Unnerstall, S. Buck, A. Jacobson, R.T. Jensen, K. Rice, C.J. Helke, B. Hoffer, T.W. Moody, G.P. Mueller, R. Quirion, J.L. Roberts, C.A. Tamminga

COOPERATING UNITS (if any)

NIGMS PRAT, NIADDK LC, NIADDK DDB, Uniformed Services Univ., Univ. Colorado, George Washington Univ., McGill Univ., Columbia Univ., Univ. Maryland

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Neuroendocrinology Unit

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

11.3

PROFESSIONAL:

8.5

OTHER:

2.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to develop an understanding of the basic regulatory mechanisms in neurons and endocrine cells which secrete peptides, and through this understanding, develop novel pharmacotherapeutic approaches and agents for manipulating peptidergic neuronal and endocrine systems. Two projects are ongoing. The first studies the regulation of biosynthesis of peptides. The primary model investigated is the neuronal and endocrine opioid system which secretes ACTH,  $\alpha$ -MSH, and  $\beta$ -endorphin- peptides all derived from a common prohormone, pro-opiomelanocortin (POMC). Our investigations indicate that regulation of POMC gene expression occurs primarily at the transcriptional level. The expression of POMC and processing enzyme genes appear to be regulated by cAMP-protein kinase A and diacylglycerol-protein kinase C mediated mechanisms. We have identified a number of putative third messenger phosphoproteins which may link cell surface receptors to transcriptional mechanisms in the nucleus.

The second investigation is focused on studies of the phencyclidine (PCP) and sigma opioid receptors and two endogenous ligands,  $\alpha$ -endopsychosin and  $\beta$ -endopsychosin, which interacts with these receptors, respectively. The peptides were isolated from extracts of porcine brain based on the ability of the compound to inhibit the binding of PCP or SKF 10,047 to brain receptors. The  $\beta$ -endopsychosin was partially sequenced and peptide fragments were synthesized. These peptides had similar biological activity to SKF 10,047. Future studies will focus on the role of these peptides in neuro and pathophysiology.

13-ET/IRP

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02139-12 ET
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pharmacology and Physiology of the Substantia Nigra and Basal Ganglia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Judith R. Walters, Ph.D., Chief, Physiological Neuropharmacology Section, Experimental Therapeutics Branch, IRP, NINCDS		
Debra Bergstrom, Ph.D., Helen Pan, Ph.D., Experimental Therapeutics Branch, IRP, NINCDS		
Barton Weick, D.V.M., PH.D., PRAT, NIGMS and Experimental Therapeutics Branch, NINCDS		
COOPERATING UNITS (if any) Pharmacology Section, Experimental Therapeutics Branch, NINCDS		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Physiological Neuropharmacology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 5.75	PROFESSIONAL: 3.75	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) 1) <u>The D-1 dopamine receptor in basal ganglia function.</u> It has previously been assumed that the dopamine receptor responsible for mediating dopamine effects on behavior is the D-2 dopamine receptor; the function of D-1 receptors has been unclear. However, we have shown that the effects of D-2 agonists on the activity of basal ganglia output neurons are significantly potentiated by coadministration of a selective D-1 receptor agonist. Moreover, effects of D-2 agonists when given alone appear dependent on endogenous dopamine providing some D-1 receptor stimulation. These studies indicate that D-1 and D-2 receptors interact synergistically to affect striatal output and demonstrate the apparent necessity for both receptors to be simultaneously stimulated for the induction of processes previously thought independently mediated by the D-2 receptor. 2) <u>Selective modulation of dopamine autoreceptor function.</u> If dopamine autoreceptors constitute a distinct subset of D-2 dopamine receptors which can be stimulated selectively by a specific agonist, such a drug might have therapeutic advantages in the treatment of tardive dyskinesia, schizophrenia and parkinsonism. We have defined the properties of two new drugs selected for potentially selectivity for dopamine autoreceptors. Both drugs, BHT920 and EMD38362, were found to be more effective agonists at dopamine autoreceptors than at postsynaptic dopamine receptors but each drug has some distinctive properties. These drugs will allow exploration of the therapeutic potential of selective dopamine autoreceptor stimulation and insight into the properties of the dopamine autoreceptors. 3) <u>Consequences of dopamine receptor denervation.</u> Neurophysiological evidence supports the idea that the consequences of stimulating D-1 dopamine receptors are altered by chronic denervation, and shows significant synergistic interactions between the dopamine receptor subtypes occur in the denervated rat. These studies and the effects of bromocriptine in the denervated rat model and in parkinsonian patients suggest that a nonselective dopamine agonist would have a greater efficacy in parkinsonism than an agonist selective for one dopamine receptor subtype.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02265-10 ET

## PERIOD COVERED

October 1, 1985 through September 1, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology, Biochemistry and Physiology of Central Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Thomas N. Chase, M.D., Chief, Pharmacology Section, Experimental Therapeutics Branch, NINCDS

A. Braun, P. Barone, H. Burrows, G. Fabbrini, J. Juncos, M. Mouradian

## COOPERATING UNITS (If any)

Department of Psychiatry, University of Maryland; Department of Psychology, Bloomsburg University; Tissue Research Center, Harvard University; Department of Psychology, Colorado University.

## LAB/BRANCH

Experimental Therapeutics Branch, IRP, NINCDS

## SECTION

Pharmacology Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

6.5

## PROFESSIONAL:

5.0

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to develop improved pharmacotherapies for central nervous system disorders based on the relation between transmitter mechanisms and clinical function. Investigations continue to focus on Parkinson's disease and Alzheimer's disease.

In Parkinson's disease, alterations in central pharmacokinetic or pharmacodynamic factors appear responsible for the marked reductions in efficacy half-life of levodopa in patients with wearing-off and especially those with on-off phenomena. The stabilization of circulating levodopa levels, with continuous levodopa or levodopa methylester infusions or sustained release formulations, rapidly eliminates wearing-off responses; on-off phenomenon diminish more slowly and less completely. D-1 and D-2 dopamine receptor mechanisms, evaluated preclinically in relation to their potential contribution to the pathogenesis of these motor fluctuation, appear complexly interactive: D-1 receptor stimulation may provide a tonic background allowing the phasic component of D-2 stimulation to become effective. In parkinsonian patients, however, administration of a selective D-1 agonist failed to influence motor function.

In Alzheimer's disease, efforts to identify transmitter system abnormalities which might provide a basis for symptomatic therapies have recently emphasized cortical peptidergic neurons, especially the somatostatin system. Spinal fluid levels of this neuropeptide are substantially below control levels; the magnitude of these reductions correlate closely with dementia severity as well as with PET determined rates of cortical glucose utilization, especially in the posterior parietal area. Nevertheless, treatment with a potent somatostatin depleting agent, designed in part to elucidate the role of this peptidergic system in dementia, significantly increased plasma growth hormone levels but had no effect on cognitive or motor function.







# ANNUAL REPORT

October 1, 1985 through September 30, 1986

## Infectious Diseases Branch

National Institute of Neurological and Communicative Diseases and Stroke

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## ANNUAL REPORT

October 1, 1985 through September 30, 1986  
Infectious Diseases Branch, IRP  
National Institute of Neurological  
and Communicative Disorders and Stroke

John Louis Sever, M.D., Ph.D., Chief

### I. RESPONSIBILITY OF THE BRANCH

The responsibility of the Infectious Diseases Branch is to carry out coordinated research programs concerned with infections which damage the human nervous system. The Branch is divided into three Sections: 1) Immunochemistry and Clinical Investigations (ICI); 2) Experimental Pathology (EP); and 3) Neurovirology and Molecular Virology (NMV). These Sections utilize the techniques of clinical investigations including human volunteers and clinical trials, experimental pathology with small laboratory animals and nonhuman primates, virology, immunology, recombinant DNA technology and gene expression, tissue culture, and electron microscopy.

### II. PROGRAM SEGMENTS

The program segments are: a) perinatal; b) acute; and c) chronic. In each segment we are concerned with: 1) etiology and diagnosis; 2) mechanisms of pathogenesis; 3) treatment; and 4) prevention.

### III. RESEARCH AREAS

The present research areas in the program segments include:

#### A. Perinatal

Investigate methods for the early diagnosis of infections which damage the CNS and study mechanism of pathogenesis and prevention. Current studies include rapid diagnostic techniques for herpes and cytomegalovirus; cofactors in AIDS; and microcephaly in congenital AIDS.

#### B. Acute

Investigate agents which may be responsible for acute neurological diseases. Current studies relate to the pathogenesis of varicella-zoster infections - varicella, shingles and Reye's Syndrome.

#### C. Chronic

Study chronic neurological diseases using combined approaches for possible infectious etiologies and mechanisms of pathogenesis. Whenever possible, explore methods for early diagnosis, treatment and prevention. Current studies include progressive multifocal leukoencephalitis, AIDS, SAIDS, post polio muscular atrophy and virus induced myelin dysgenesis.

#### IV. SECTION ACTIVITIES

##### A. Section On Immunochemistry and Clinical Investigations (ICI)

###### 1. Perinatal

###### a. Early Diagnosis

We have developed a new test for rapid typing of herpes simplex virus (HSV) using monoclonal type specific antibody and biotin-avidin reagents. The test involves capturing the virus on polystyrene plates coated with rabbit polyclonal anti-HSV antibody. Biotin-linked monoclonal anti-HSV type antibody is used as the detecting antibody. The captured HSV antigen is then detected by reaction with streptavidin enzyme conjugate. The test has 100% sensitivity and specificity compared to typing performed by monoclonal antibody fluorescent antibody technique and restriction endonuclease analysis of HSV genome. The advantages of this test are: a) it requires relatively short time, b) it uses nonradioisotopic reagents, and c) it has a potential for automation when large samples have to be analyzed.

We have made progress in developing a rapid test for the detection of cytomegalovirus (CMV). The test involves tissue culture isolation of the virus for at least 48 hours and immunofluorescence staining for virus expressed protein antigen(s) with specific monoclonal antibody. Amplified detection system involving biotin conjugated antimouse antibody and avidin fluorescein conjugate increases sensitivity and specificity.

###### b. Cytomegalovirus

Longitudinal studies of progressive CNS and eye damage in children with congenital CMV infections are being conducted. We are investigating effect of intrauterine CMV infection of pregnant rhesus monkey mothers and their babies. The immunological and virological studies of these mother-baby pairs are performed at weekly intervals. These studies include determination of T lymphocyte subsets alterations, total immunoglobulin and specific antibody responses, lymphocyte proliferation with mitogens and CMV antigen, cytotoxic function along with virus isolation.

###### c. AIDS

We are studying pregnant women who have AIDS and the pathogenesis, immunological changes and cofactors involved in the development of microcephaly which occurs in up to 50% of cases of congenital AIDS.

###### d. Collaborative Study

We are completing two reports from the Collaborative Perinatal Study. The first is on Toxoplasmosis During Pregnancy and the second is an analysis of Maternal Infections with papilloma viruses. A third report will present data for Abnormal and Matched Control Pregnancies. These studies will complete the "Core" investigations of this project. The clinical data and serum specimens are maintained as a national resource for NIH and outside groups. Proposals for use of the data are reviewed by a NINCDS committee and when approved, the information and specimens are released. Clinical data is on six computer tapes and the serial serum specimen from 58,000 pregnancies are in the Serum Center of the IDB. At present we are supplying specimens for collaborative

studies being conducted with the NICHD, NIAID, University of California and the CDC.

## 2. Acute

### a. Clinical Studies - Reye's Syndrome

Clinical investigations of Reye's Syndrome patients are being conducted at NIH. We have investigated the metabolism of aspirin by children who have survived Reye's Syndrome and controls. We are now studying the patient's viral-immune responses for influenza A, B, and varicella-zoster.

### b. Animal Studies - Varicella-Zoster

Studies are being conducted using monkeys with simian varicella as a model for human varicella, shingles and possibly for Reye's Syndrome. Cellular immune responses in acute and recurrent infections are being studied with monoclonal antibodies to various cell types using the cell sorter. Latency of the virus is being investigated with hybridization and other techniques. (With the NV and EP Sections).

## 3. Chronic

### a. AIDS

We are conducting a longitudinal study of homosexual men at high risk for AIDS in Los Angeles. The study is in its 4th year. Some of the individuals have developed AIDS. We are investigating the immunological responses and cofactors which are responsible for some of those patients developing clinical AIDS while others remain asymptomatic. At NIH we are studying the pathogenesis of the neurological findings in AIDS, and we are admitting patients with unusual neurological symptoms and individuals who have HTLV III antibody and only neurological findings. We are also investigating the adoption of the HTLV III virus to monkey cell lines and other approaches for the development of an AIDS virus animal model with clinical findings similar to those which occur in human AIDS. (With NV and EP Sections).

### b. SAIDS

Cellular and humoral immunological studies of Simian Acquired Immuno-deficiency Syndrome (SAIDS), and Acquired Immunodeficiency Syndrome (AIDS), are being conducted. The SAIDS retrovirus are similar to the human AIDS virus and provide an opportunity to investigate the pathogenesis of this disease. In SAIDS, the monoclonal OKT3 antigen has not been found to be present on either normal or SAIDS infected monkey lymphocytes. The NEN-Lyt 9.6 monoclonal antibody was found to be a better reagent than OKT-11 in detecting the E-rosetting marker on monkey lymphocytes. These findings will be applicable to all other studies in which lymphocyte markers are needed to investigate immunological diseases in nonhuman primates. Studies are in progress to determine the retrovirus susceptibility of various cells in SAIDS and AIDS. Continuous cell lines of various lineages (Pre T, late T and B cells) have been studied for specific receptor as T4/T8 and sensitivity to retrovirus infection. Serological tests have been developed for a variety of human and nonhuman retrovirus. Serum from several patient populations have been tested. We have found an association of HTLV-I and tropical spastic paraparesis. These patients are being further studied to determine in vitro immunological responses. The neuromuscular complications of monkeys with SAIDS and patients with AIDS have been actively studied. Polymyositis has

been seen in both SAIDS and AIDS and several neuropathies in AIDS patients. The role of these retroviruses in nerve and muscle involvement is being studied. (With NV and EP Sections).

c. MS, SSPE, and Polymyositis

In our studies of multiple sclerosis (MS), subacute sclerosing panencephalitis (SSPE), we found that a new silver staining technique for protein in acrylamide gel electrophoresis has greatly increased our ability to detect oligoclonal bands in the CSF. Sera and CSF from twin pairs, one of which has multiple sclerosis, have been studied for presence of viral antibodies to measles, rubella, vaccinia and mumps. Increased levels of rubella and vaccinia antibodies were found in MS affected individual when CSF serum antibody levels were compared. Serum and CSF measles antibody titer were high in DW2 twin but was not associated with clinical disease. Diagnostic significant level of measles antibody in serum and CSF of patients with SSPE have been established. Immunoglobulin subclasses have been determined in serum and CSF of SSPE patients. IgM was not detected, IgG<sub>1</sub> and IgA subclass were elevated. IgG<sub>2</sub> and IgG<sub>3</sub> were detected but not increased. IgG<sub>1</sub>, was also the prominent IgG class in normal individuals. The immunological markers on lymphocytes and the non-specific responses to SSPE patients did not differ from the controls.

Muscle biopsies of autoimmune mice with a genetically determined lupus-like disease were examined for an associated inflammatory myopathy. Tubular aggregates in abundance were found in male mice and their presence was correlated with increased interferon. Interferon causes tubuloreticular formations in many cells of patients with autoimmune or viral diseases. These findings suggest that interferon can also cause cytoskeletal abnormalities of the sarcoplasmic reticulum.

d. PPMA, ALS, PML - Clinical and Laboratory Studies

Clinical, immunological and virological studies of late post-poliomyelitis muscular atrophy (PPMA), ALS, PML, polyneuropathies, polymyositis and metabolic myopathies are being conducted. Patients with Progressive Multifocal Leukoencephalopathy are being studied prospectively for immune defects specific for the etiologic agent, JC virus, comparison of in situ hybridization and antigen detection for diagnosis using brain biopsy, and a comparison of CT scanning and MRI scanning as possible diagnostic procedures.

We have performed several clinical, immunological and virological studies involving patients with neuromuscular and demyelinating diseases. Specifically, we have defined the clinical spectrum of new symptoms and signs that occur many years later in patients with prior paralytic poliomyelitis. We have also completed a 12-year follow-up study of patients with new symptoms which established the rate of progression on a year-to-year basis. The pathogenetic mechanisms of this disease were investigated with: a) a detailed virological and immunological screening in the serum and CSF, b) histological studies in their newly affected muscles, c) electrophysiological investigation including single fiber EMG, and d) epidemiological survey of 2,000 previously affected patients to establish the frequency of the disease. We found that in post-polio patients there appears to be peripheral disintegration of the distal axons of the surviving motor neurons. The status of the upper motor neurons in post-polio patients was also studied using PET

scan and  $^{18}\text{F}$ -2-deoxy-D-glucose and the findings were compared with the pattern seen in ALS patients.

We observed that the metabolic activity of the motor sensory cortex is normal in post-polio motor neuron diseases whereas in ALS patients there appears to be widespread hypometabolism involving the motor and paramotor cortical regions. We have also completed an experimental therapeutic trial in ALS patients using recombinant DNA interferon. This was ineffective in arresting the progression of the disease or changing the metabolic status of the motor cortex, as studied with the PET scan.

We have also studied immunologically, immunocytochemically, and neurophysiologically patients with peripheral neuropathies. We have identified that the monoclonal IgM from patients with paraproteinemic neuropathies is an antibody against either the myelin associated glycoprotein (MAG) or against different glycolipids and gangliosides, the identity of which was defined. Thus, glycolipids can be new, strong antigens in the pathogenesis of patients with neuropathy. Using immunochemical techniques we have also determined the nature of amyloid protein in patients with sporadic amyloid polyneuropathy as being related to immunoglobulin light chain. We studied the mechanism and importance of proprioceptive input for the motor control in a group of 14 de-afferented patients with ataxic sensory neuropathy but normal strength, and defined the clinical spectrum of this neuropathy.

Investigating for mechanisms of demyelination in the CNS and seeking possible interaction of myelin supporting cells with cells of the lymphoid organs, we found that the thymic hormone thymosin beta  $^4$  is a shared antigen between human oligodendrocytes and macrophages or other  $1\text{a}^+$  cells of the lymphoid system. This supports the presence of an immune link between activated macrophages and oligodendrocytes, which can help us to understand the mechanism of destruction of oligodendrocytes in human immune demyelinating diseases of the CNS.

Other studies of neuromuscular diseases include: a) the establishment of the viral model of polymyositis in monkeys using a well characterized (by IDB virologists) new retrovirus D, which provides evidence that the virus directly or via infected cells is responsible for the muscle damage, b) the identification of polymyositis in patients with AIDS and the pathogenetic implications of the HTLV-III retrovirus in the muscle damage, and c) the identification of a metabolic defect due to carnitine deficiency in the muscle of patients with cystinosis. The latter finding prompted an ongoing experimental therapeutic trial with carnitine in an effort to increase the strength of these patients.

#### e. Virus Induced Myelin Dysgenesis

Our in vivo studies have shown that although Border Disease (BD) virus infects many cell types in the fetal sheep CNS (granule cell neurons, oligodendrocytes, a rare astrocyte and endothelial cells) it affects the oligodendrocyte at a critical stage in development causing a significant delay in myelinogenesis. In the acute infection at mid-gestation the earliest marker for oligodendrocytes, Gal-C, and a latter differentiation antigen, myelin basic protein (MBP) are absent. This is in comparison to full Gal-C and partial MBP expression in age matched controls. This delay in synthesis

of myelin related markers is maintained in the persistent infection where a differential reduction in myelin associated glycoprotein (MAG), MBP and proteolipid protein are noted.

Our in vitro studies have shown that multiple neural cell types are infected. Our current in vitro studies are focused on determining how BD virus interferes with differentiation of oligodendrocytes and astrocytes. Proliferating cells which are morphologically similar to cells described as the 0-2A precursor glial cell in the rodent system expressing only the cell surface marker A2B5 have been maintained and when these cells are cultured in 5% horse serum they quickly began to express vimentin and GFAP and acquire the morphology of astrocytes. We are now studying the affects of BD virus on these cultures using immunocytochemistry and cell sorting.

## B. Section On Experimental Pathology (EP)

### 1. Perinatal

#### a. Rhesus Cytomegalovirus RCMV

We have shown that rhesus monkey fetuses inoculated intraamniotically in the first trimester or intracerebrally in the second trimester develop congenital disease. Lesions are restricted to the CNS and consist of ventricular dilatation, leptomenigitis, choroid plexitis, ependymitis and focal encephalitis. Our model will allow investigators to study the pathogenesis of CNS changes following RCMV infection. (With ICI Section).

#### b. Group B Streptococci (GBS)

Our studies of perinatal infection with Group B streptococci (GBS) using a rhesus monkey model are in their final stages. Studies to determine the efficacy of hyperimmune human IgG in treating GBS infected rhesus infants nears completion. Results indicate that severe infections with GBS can be treated successfully with hyperimmune immunoglobulin. Our observations indicate that newborn rhesus are most susceptible to GBS infection following ingestion of the organism. Although pulmonary infection has been proposed as the portal of entry, infant rhesus inoculated intraamniotically the day before delivery invariably have GBS in the digestive tract but only rarely is the organism present in the lungs.

### 2. Acute

Studies are in progress to evaluate whether Simian Varicella (SV) virus, like human varicella virus, is able to establish a latent infection in the natural host; i.e., rhesus monkeys. We are also investigating the Simian SV in relation to Reye's Syndrome. (With NV and ICI Sections).

### 3. Chronic

a. Neuro-oncogenic studies continue with owl monkeys inoculated intracerebrally with JC virus MAD 1 and 4 human polyomavirus (with the NMV Section).

b. Studies of Simian Retrovirus Type I (SRV I) and Simian T Lymphotropic Virus Type III (STLV III) (the etiological agents of Simian AIDS) are

continuing. One major emphasis of our studies is directed toward identifying which host cell types are susceptible to infection with SRV I and STLV III. We have shown peripheral blood mononuclear cells to be infected only transiently with STLV III. In other infections, including AIDS, macrophages have been proposed as reservoirs for virus replication. We are currently investigating the role of macrophages in SAIDS and AIDS.

### Section On Neurovirology & Molecular Virology (NMV)

#### Perinatal

None.

#### Acute

Studies have been initiated to determine the relative virulence of a new strain of simian varicella virus for several species of monkeys. Restriction enzyme mapping is being used to characterize the viral genome and its relation to other strains of varicella from humans and monkeys. These studies have been initiated for purposes of studying reactivation of latent varicella infection and its attendant neurological complications. Attempts are being made to develop a model for Reye's Syndrome. (With the EP Section).

#### Chronic

##### a. JC Virus - PML

We have completed the assessment of using biotin labeled viral DNA probes and *in situ* DNA hybridization for the laboratory diagnosis of JC virus infection in PML. This new methodology shows significantly better specificity over immunocytochemical techniques. Analysis has now been done on formalin fixed, paraffin embedded brain tissues. The study of the molecular control of JCV in human brain has revealed that nucleotide sequences in the regulatory region of the viral genome serve as transcriptional enhancer elements only in glial cells. A comparison of a rat neuronal sequence which shares homology to the JC enhancer region, however, was not functional. Therefore, the cell specific control of virus infection is probably related to viral proteins and cellular factors as yet unidentified. Two new isolates of JC virus have been made. One is derived from a PML brain and the other from an owl monkey glioblastoma cell line. In both cases, genetic alterations have taken place which may reflect the ability of JCV grown in brain to undergo gene rearrangements. Future studies in these naturally occurring genomic variants could lead to understanding the host control of JCV infection. (With EP Section).

##### b. SHF

Differences between acute and persistent infections are being sought via use of the patas monkeys - simian hemorrhagic fever virus model. Virological and immunological techniques are being used to determine the mechanism of elimination of persistent SHF virus infection by superinfection. Physical-chemical differences between acute and persistent strains of SHF virus are being sought by immunological and biochemical techniques, and work is in progress to characterize the virus for purposes of classification. Cellular immunology techniques are being used to elucidate the cellular interactions involved in restricting the immune response and maintaining tolerance of persistent SHF virus infection. Immune enhancement leading to death is being studied in macaque monkeys. (With the EP Section).

### c. SAIDS

We and others have shown that strains of a type D retrovirus related to Mason-Pfizer monkey virus (MPMV) called SAIDS retrovirus type 1 or 2 (SRV-1 or SRV-2), are the cause of simian acquired immunodeficiency syndrome SAIDS, a frequently fatal disease of macaque monkeys which in many respects resembles human AIDS. Although SAIDS and AIDS have many common clinical and pathological features, the mechanism of immunosuppression for each disease is probably different. The cell types involved and the mechanism by which SRV-1 exerts its immunosuppressive effect is currently being studied by immunological virological and molecular biological techniques. (With EP and ICI Sections).

Recently a retrovirus, called simian T-lymphotropic virus type III (STLV-III), which closely related antigenically and morphologically to HTLV-III/LAV was reported to be isolated from monkeys with an AIDS-like immunosuppressive disease. We have succeeded in isolating a similar virus from asymptotically infected African green monkeys. Work is in progress to characterize our isolate and to determine whether it can produce an AIDS-like disease in macaque species of monkeys. (With EP Section).

Virological and immunological support has been provided for the AIDS studies being performed by investigators from the ICI Section.

### d. Varicella-Zoster

A new strain of simian varicella-zoster virus has been isolated which has variable virulence for different species of nonhuman primates. It produces a mild chickenpox-like illness in rhesus monkeys, but a more severe, frequently fatal disease in patas and African green monkeys. We hope to develop an animal model of human chickenpox and shingles by use of this new isolate. (With the EP Section).

## V. Findings

### A Perinatal

#### 1. Congenital Disease Produced With Rhesus Cytomegalovirus In Rhesus Monkeys

Rhesus fetuses infected with rhesus CMV (IA or IC) developed CNS malformations. This is the first example of a teratogenic effect produced by CMV in animals other than man. Studies of pathogenesis, treatment and prevention are now possible.

#### 2. Incidence Of Clinical Infections In Pregnant Women

Over 90% of women had significant clinical infections during pregnancy. Some of these infections were important to the development of the fetus.

#### 3. Approaches Used For The Detection Of Infectious Agents As Human Teratogens

Several different approaches can now be used to detect teratogenic agents. New methods for assay provide valuable tools to prevent birth defects.



#### 4. Delayed Manifestations Of Congenital Rubella

Late effects of congenital rubella include diabetes, abnormal thyroid function and retarded growth. These effects do not appear until the second or third decade of life.

#### 5. Immunologic Findings In A Case Of Congenital CMV Compared To Infants With AIDS

We demonstrated that T4/T8 ratio can be reversed in congenital CMV, resembling AIDS. Later however, these changes revert to normal.

#### B. Acute

##### 1. Polymyositis Occurs In Monkeys With Type D Retrovirus

Polymyositis occurred in 11 of 25 monkeys which died of AIDS. This disease closely mimicked human polymyositis and provides a model for the study of human viral induced polymyositis.

#### C. Chronic

##### 1. Differences Among Isolates Of SHF Virus

Strains of SHF virus were identified to be in two main groups; those that produce acute infections and those that cause persistent infection. These groups are related structurally and biochemically but biological differences were found in cell types which are infected, types of disease produced, and serological differences were demonstrated.

##### 2. Elimination Of Persistent Infection With SHF Virus

Persistent SHF infection was cleared by superinfection with a related, acute strain of SHF virus. This is important for the control of this disease in monkeys, and it provides new information on immunological control of persistent infections.

##### 3. Animal Models Of Retrovirus Infections In Relation To AIDS

Comparisons of the diseases produced in monkeys by retroviruses showed considerable similarities to AIDS in humans.

##### 4. Viral Antibodies In Twins With Multiple Sclerosis

Increased levels of antibodies to measles, rubella and vaccinia were found in patients with MS and twins. Increased measles antibody was associated with tissue type DW-2.

##### 5. Comparison Of Detection Of Oligoclonal IgG Bands In CSF

The silver stains were found to identify oligoclonal bands earlier and more frequently than other methods of staining.

6. Absence Of HTLV-III Antibody In Blood Donors And Recipients In India

Studies of samples from blood donors and recipients (renal transplants) in India showed no evidence of AIDS antibody.

7. Late Effects Of Poliomyelitis

The clinical syndrome of late post-poliomyelitis muscular atrophy (PPMA) have been described and possible pathogenetic mechanisms of the disease were investigated. A long-term follow-up study was completed and the rate of progression of post-polio weakness was estimated.

8. Human Thymic Hormones Present In Certain Lymphocytes, Lymphoid Organs And Oligodendrocytes

The demonstration of receptors for human thymic hormones in certain lymphocytes, lymphoid organs and oligodendrocytes suggests a possible link between the lymphoid system and oligodendrocytes in the brain.

9. Identification Of New Antigens In The Peripheral Nerves Of Patients With Paraproteinemic Polyneuropathies

Monoclonal IgM antibody against peripheral nerve MAG, glycolipids and ganglioside was demonstrated in patients with paraproteinemic polyneuropathies. This appears to be important in the pathogenesis of the disease. The CSF and clinical findings in the disease were studied in detail.

10. Amyloid In Patients With Sporadic, Non Plasma Cell Dyscrasia Amyloid Polyneuropathy Is Often Related To Immunoglobulin Light Chains

Although patients may show no plasma cell dyscrasia, amyloid can be of immunoglobulin origin, perhaps produced by plasma cells or B cells in chronic inflammatory regions. These patients may now be treated with immunosuppression.

11. Children With Cystinosis And Renal Fanconi Syndrome Have Carnitine Deficiency In Plasma And Muscle

These findings have suggested that these children can be treated with carnitine. Clinical studies are in progress.

12. Polymyositis Present In Patients With AIDS

Several neuromuscular complications including chronic or acute polyneuropathies of the inflammatory type and inflammatory myopathies were described and studied in patients with AIDS. Polymyositis was identified in 2 AIDS patients and the role of HTLV-III in the pathogenesis of the muscle disease was investigated. The role of HTLV-III as a causative agent of demyelination neuropathies is also being studied.

13. Laboratory Diagnosis Of PML In Patients

Formalin fixed, paraffin embedded PML brain tissues were analyzed by in situ hybridization using biotin labeled JCV DNA probes which confirmed the clinical diagnosis of PML.

14. Comparison Of Molecular Assays To Detect JCV DNA Or Antigen

Analysis of in situ DNA hybridization was compared with viral antigen detection in the laboratory diagnosis of PML and found to offer significant advantage of specificity identifying JCV as the etiologic cause of this disease.

15. JCV DNA Possesses Transcriptional 'Enhancer' Sequences

Mechanisms of viral gene regulation in human brain cells were examined. Using transcriptional assays it was shown that JCV enhancer sequences function only in glial cells.

16. JCV DNA Replication Origin Can Bind SV40 T Protein

The JCV DNA replication origin can use a trans acting protein made by SV40, the T protein, in human kidney cells only if nucleotide sequences next to the origin serve as a T protein binding site. This defines a genome region of JCV DNA which may have functional importance initiating infection.

17. JC Virus Is Released From A Transplantable Glioblastoma Cell Line

JC Virions are produced from a tumor cell line of owl monkey glioblastoma cells with a structurally altered T protein. This indicates genetic alterations in the genome which could explain the ability of this strain of JCV to grow in simian brain cells.

18. Human Fetal Brain Synthesize A Cellular Onc Gene Product

A cellular, nuclear oncogene, p53, was identified in human fetal glial cells which is made during early brain development. Its presence may be important for differentiation of glial cell populations.

CONTRACT NARRATIVE

Infectious Diseases Branch, IRP, NINCDS

Fiscal Year 1986

Microbiological Associates: (N01-NS-3-2316)

TITLE: Development and Delivery of Antigen, Antisera, and Viral Diagnostic Reagents.

Contractor's Project Director: Dr. David A. Fuccillo

Current Funding: \$175,000.00

Objectives: This is a service contract to provide research reagents for studies of neurological diseases which may have infectious etiologies and special investigations of polyomaviruses, AIDS and simian AIDS (SAIDS).

Major Findings: Viral diagnostic reagents have been provided for herpes viruses types I and II, cytomegalovirus, measles, rubella, influenza, and varicella viruses. These antigens are used in an attempt to identify the etiology of neurological infections. Evaluation of reagents and materials required to produce successful enzyme-linked immunosorbent assays (ELISA) was accomplished. Reagents for acquired immune deficiency syndrome (AIDS) are being developed to study this highly fatal disease. A similar outbreak of simian AIDS-like disease (SAIDS) has occurred in rhesus monkeys. Antigens for indirect immunofluorescence have been prepared for HTLV-I, HTLV-III, SRV-I and STLV-III. Sera from selected monkey and human populations have been evaluated. Reagents to study rhesus monkey CMV and its relationship to SAIDS have been prepared. Large quantities of a retrovirus are being prepared for comparison studies to be done against a similar virus found in SAIDS. Reagents for ELISA tests have been developed for the JC papovavirus. Reagents have been prepared for studies on the molecular genetics of the BK and JC virus.

Preparation and analysis of plasmids DNAs have been done on four recombinant DNA constructions. Two of these plasmid DNAs contain eucaryotic gene sequences which code for proteins expressed in human fetal brain; namely the p53 phosphoprotein involved in cell cycle and transformation and the glial fibrillary acidic protein (GFAP), the structural intermediate filament of astroglial cells. Two other plasmids contain segments of the viral genome of the neurotropic human polyomavirus JCV. For each of these DNA constructions, large volumes of plasmid carrying host *E. coli* bacteria were grown and the plasmid DNA was isolated. Following purification from host DNA, these plasmids were analyzed by restriction endonuclease mapping to assure that alterations had not taken place during plasmid growth.

Publications:

Shekarchi, I.C., Sever, J.L., Nerurkar, L. and Fuccillo, D.: Comparison of enzyme-linked immunosorbent assay with enzyme-linked fluorescence assay with automated readers for detection of rubella virus antibody and herpes simplex virus. J. Clin. Microbiol., 21(1): 92-96, 1985.

Fuccillo, D.A., Shekarchi, I.C. and Sever, J.L.: Rapid viral diagnosis. In Manual of Clinical Laboratory Immunology, Third Edition, N.R. Rose, H. Friedman, J.L. Fahey (eds.), American Society for Microbiology, Washington, D.C., 1986, Chapter 74, pp. 489-496.

Fuccillo, D.A. and Sever, J.L.: Measles, mumps, and rubella. In Clinical Virology Manual, Elsevier Science Publishing Co., Inc., New York, pp. 437-449, 1986.

# CONTRACT NARRATIVE

Infectious Diseases Branch, IRP, NINCDS

Fiscal Year 1986

Meloy Laboratories, Inc.: (N01-NS-5-2377)

Title: Isolated Housing and Care of Animals Used in Studies of Infectious Diseases of the Central Nervous System.

Contractor's Project Director: Dr. Jere M. Phillips

Date Contract Initiated: 16 May 1986

Current Annual Level: \$218,346.00

Objectives: The contract provides isolated housing and care for laboratory rodents and a colony of nonhuman primates consisting of several genera. The animals are on experimental studies directed by written protocols. They require monitoring daily for clinical signs of disease. Biological specimens are collected as prescribed by protocols. The aims of the contract are to provide the facilities which permit animal studies that are judiciously planned to be humanely carried out. Animal studies carried out for the IDB, NINCDS are designed to investigate the etiology, pathogenesis, early diagnosis, treatment and prevention of both known and suspected infectious diseases of the nervous system.

Methods Employed: Animals are quarantined, conditioned and screened for pre-existing antibodies to agents under investigation. Seronegative animals are inoculated by a variety of routes. The infected animals are then held in individual isolation units, monitored and tested as directed in written protocols.

Major Findings: This contract satisfactorily provides housing and care for most of the laboratory animals needed for research in the Infectious Diseases Branch. Animals are used in a number of studies of the infections of the central nervous system (CNS). Experimental animals which become sick are promptly identified and supportive therapy instituted. The investigators on the contract provide overall daily clinical care for the entire colony, with strict isolation procedures carried out at all times. The Contractor's Project Director makes modifications of studies when necessary to achieve the overall goals of the contract. The facility is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). The accredited guidelines are designed to assure proper and humane care of all laboratory animals. These guidelines are strictly adhered to by facilities funded under this contract.

Publications: None. All publications from this contract are listed in each section of the Infectious Diseases Branch Annual Report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-00402-30-ID

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Perinatal Infections Causing Damage to the Children in the CPP

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI John L. Sever, M.D., Ph.D.	Chief	IDB, IRP, NINCDS
David L. Madden, D.V.M., Ph.D	Veterinary Director	IDB, IRP, NINCDS
Others: Jonas Ellenberg	Chief	BFSB, IRP, NINCDS
Nancy Madden	Microbiologist	IDB, IRP, NINCDS
Dorothy M. O'Neill	Clinical Nurse	IDB, IRP, NINCDS

COOPERATING UNITS (if any)

BFSB, IRP, NINCDS

LAB/BRANCH

Infectious Diseases Branch

SECTION

Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The purpose of this study is to determine insofar as possible the role of perinatal infections in the production of fetal damage. To accomplish this, clinical data and a large number of serial serum specimens were obtained from the 58,000 women and their children in the Collaborative Perinatal Project. A number of reports and publications have come from the study. During this year papers have been published summarizing approaches used by the study and the incidence of clinical infections in the study population. Current efforts have been focused on completing the analysis and publication of the two remaining major studies from the project: 1) Toxoplasmosis and Fetal Damage, 2) Papilloma Viruses And Fetal Damage, and 3) The Study Of The Pregnancies Of Abnormal Children And Matched Controls. We are also supplying clinical data and serum specimens from the Collaborative Project for several other studies including: NICHD Study On Maternal Diabetes; NIAID Investigations Of AIDS; University of California, Berkeley studies of cancer and thyroid disease; and a study with the CDC on fetal abnormalities caused by parvovirus infections.

15-IDB/IRP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01-NS-02532-04-ID</b>				
PERIOD COVERED October 1, 1985 through September 30, 1986						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Study of AIDS and SAIDS Neurological Findings and Etiology</b>						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 20%; vertical-align: top;">           P.I.            Others:         </td> <td style="width: 60%; vertical-align: top;">           J.L. Sever, M.D., Ph.D.            M. Gravel, Ph.D.            W.T. London, D.V.M.            S.A. Houff, M.D.            D.L. Madden, D.V.M., Ph.D.            M.C. Dalakas, M.D., Ph.D.            G. Elder, M.D.            L. Nerurkar, Ph.D.            M. Monzon, Ph.D.            N. Goldblum, Ph.D.         </td> <td style="width: 20%; vertical-align: top;">           Chief,            Research Microbiologist            Veterinary Director            Neurologist            Veterinary Director            Medical Staff Fellow            Medical Staff Fellow            Special Expert            Visiting Scientist            Fogarty Scholar         </td> <td style="width: 10%; vertical-align: top;">           IDB, TRP, NINCDS            IDB, TRP, NINCDS            IDB, TRP, NINCDS            IDB, TRP, NINCDS            IDB, TRP, NINCDS            IDB, TRP, NINCDS            IDB, TRP, NINCDS            IDB, TRP, NINCDS            IDB, TRP, NINCDS            IDB, TRP, NINCDS         </td> </tr> </table>			P.I. Others:	J.L. Sever, M.D., Ph.D. M. Gravel, Ph.D. W.T. London, D.V.M. S.A. Houff, M.D. D.L. Madden, D.V.M., Ph.D. M.C. Dalakas, M.D., Ph.D. G. Elder, M.D. L. Nerurkar, Ph.D. M. Monzon, Ph.D. N. Goldblum, Ph.D.	Chief, Research Microbiologist Veterinary Director Neurologist Veterinary Director Medical Staff Fellow Medical Staff Fellow Special Expert Visiting Scientist Fogarty Scholar	IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS
P.I. Others:	J.L. Sever, M.D., Ph.D. M. Gravel, Ph.D. W.T. London, D.V.M. S.A. Houff, M.D. D.L. Madden, D.V.M., Ph.D. M.C. Dalakas, M.D., Ph.D. G. Elder, M.D. L. Nerurkar, Ph.D. M. Monzon, Ph.D. N. Goldblum, Ph.D.	Chief, Research Microbiologist Veterinary Director Neurologist Veterinary Director Medical Staff Fellow Medical Staff Fellow Special Expert Visiting Scientist Fogarty Scholar	IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS IDB, TRP, NINCDS			
COOPERATING UNITS (if any) Section of Neurovirology, IDB, IRP, NINCDS Section of Experimental Pathology, IDB, IRP, NINCDS						
LAB/BRANCH Infectious Diseases Branch						
SECTION Immunochemistry and Clinical Investigations						
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892						
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:				
8.7	4.9	3.8				
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews						
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p> <u>Rhesus monkeys</u> infected with <u>SAIDS retrovirus type 1 (SRV-1)</u> frequently develop a fatal <u>immunosuppressive disease</u> with many of the clinical and pathological features of <u>human AIDS</u>. HTLV-III/LAV, the causative agent of AIDS, has been found to have a narrow host cell range, replicating preferentially in T4<sup>+</sup> lymphocytes. SRV-1 has a much broader host cell than HTLV-III/LAV, infecting such cell types as fibroblasts, kidney cells and B and T lymphocytes. <u>Inverted T4/T8 lymphocyte</u> ratios have not been seen in animals with SAIDS, regardless of their disease stage. These results suggest that SRV-1 exerts its immunosuppressive effect by a mechanism different from HTLV-III/LAV.         </p> <p> <u>Polymyositis</u> was a frequent complication of rhesus monkeys who experienced fatal SAIDS. Eleven of 25 fatally infected animals showed clinical, histologic and/or histochemical evidence of polymyositis. Many of the clinical, histological and histochemical features of the polymyositis seen in these animals resembled those reported for virus-induced human polymyositis. Thus, further study of this model appears warranted.         </p> <p>           Patients with <u>HTLV-III/LAV antibody</u>, with or without AIDS or ARC, continue to be evaluated for possible HTLV-III/LAV related disease of the central or peripheral nervous systems.         </p>						
16-IDB/IRP						



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-01985-15-ID

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Perinatal and Neurological Diseases - Viral and Nonviral Antigens or Antibodies \*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: D. L. Madden, D.V.M., Ph.D.	Veterinary Director,	IDB, IRP, NINCDS
Others: J. L. Sever, M.D., Ph.D.	Chief	IDB, IRP, NINCDS
G. Elder, M.D.	Med. Staff Fellow	IDB, IRP, NINCDS
L. Nerurkar, Ph.D.	Special Expert	IDB, IRP, NINCDS
D. Budzko, Ph.D.	Special Expert	IDB, IRP, NINCDS
S. Deshmane, Ph.D.	Visiting Fellow	IDB, IRP, NINCDS
B. Freij, M.D.	Visiting Fellow	IDB, IRP, NINCDS
M. Ceroni, M.D.	Guest Researcher	IDB, IRP, NINCDS

## COOPERATING UNITS (if any)

Microbiological Associates, Inc.  
University Medical School, Milano, Italy  
University of South Alabama, Mobile, Alabama

LAB/BRANCH  
Infectious Diseases Branch

## SECTION

Immunochemistry and Clinical Investigations

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.5

## PROFESSIONAL:

1.75

## OTHER:

.75

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

At the Clinical Center, NIH, we have been studying patients who have recovered from Reye's syndrome and controls. We have found that the metabolism of aspirin in these patients is not abnormal. Immune responses however are abnormal for several viruses. These findings are being extended.

Techniques to improve the rapid viral diagnosis of acute and persistent infections which affect the CNS received special emphasis. We have developed a technique to identify herpes virus in clinical specimens within 24-36 hours. These isolates can be immediately typed using a capture technique which employs polyclonal antibody and a specific second monoclonal antibody. Longitudinal studies of a child with a severe neurological involvement following CMV infection has been reported. After 3 years, the cellular immune state of the child improved and viremia and uremia disappeared. In vitro studies of immunosuppressive drugs on permissiveness and growth of CMV in tissue culture cells indicated that the drugs did not enhance the virus replication. We have studied a large population of adult students with congenital rubella. Almost 20% of the students have developed diabetes. Our results indicate that CMV infection is not causatively associated with diabetes mellitus, however IgM rubella antibody studies are in progress. Sera and CSF were studied from 24 MS twin pairs (one had MS, the other was normal). Measles and rubella virus antibody levels were elevated in the MS affected individuals. Serum and CSF antibody levels were elevated in DW2 individuals, but DW2 and antibody levels were not associated with clinical symptoms. We have established critical levels for measles antibodies in the serum and CSF of SSPE patients when measured by ELISA techniques. This will be useful for physicians in differential diagnosis.

\*Formerly "Viral and Nonviral Antigens or Antibodies in Perinatal and Neurological Diseases"

17-IDB/IRP

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01-NS-02038-14-ID</b>			
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>					
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Combined Clinical, Viral and Immunological Studies of Peripheral and CNS Diseases</b>					
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <b>PI: M. C. Dalakas, M.D.</b>  <b>Others: J.L. Sever, M.D., Ph.D.</b>  <b>M. Hallett, M.D.</b>  <b>G. Elder, M.D.</b>  <b>P. Plotz, M.D.</b>  <b>G.H. Pezeshkpour, M.D.</b>  <b>G. Di Chiro, M.D.</b>  <b>G. Sanes, M.D.</b> </td> <td style="width: 50%; vertical-align: top;"> <b>Senior Staff Fellow</b>  <b>Chief,</b>  <b>Clinical Director,</b>  <b>Neurologist</b>  <b>Rheumatologist</b>  <b>Neuropathologist</b>  <b>Neuroradiologist</b>  <b>Neurophysiologist</b> </td> <td style="width: 50%; vertical-align: top;"> <b>IDB, IRP, NINCDS</b>  <b>IDB, IRP, NINCDS</b>  <b>OCB, NINCDS</b>  <b>IDB, IRP, NINCDS</b>  <b>ARB, IRP, NIMSD</b>  <b>AFIP, Wash. D.C.</b>  <b>OD, IRP, NINCDS</b>  <b>OCB, IRP, NINCDS</b> </td> </tr> </table>			<b>PI: M. C. Dalakas, M.D.</b> <b>Others: J.L. Sever, M.D., Ph.D.</b> <b>M. Hallett, M.D.</b> <b>G. Elder, M.D.</b> <b>P. Plotz, M.D.</b> <b>G.H. Pezeshkpour, M.D.</b> <b>G. Di Chiro, M.D.</b> <b>G. Sanes, M.D.</b>	<b>Senior Staff Fellow</b> <b>Chief,</b> <b>Clinical Director,</b> <b>Neurologist</b> <b>Rheumatologist</b> <b>Neuropathologist</b> <b>Neuroradiologist</b> <b>Neurophysiologist</b>	<b>IDB, IRP, NINCDS</b> <b>IDB, IRP, NINCDS</b> <b>OCB, NINCDS</b> <b>IDB, IRP, NINCDS</b> <b>ARB, IRP, NIMSD</b> <b>AFIP, Wash. D.C.</b> <b>OD, IRP, NINCDS</b> <b>OCB, IRP, NINCDS</b>
<b>PI: M. C. Dalakas, M.D.</b> <b>Others: J.L. Sever, M.D., Ph.D.</b> <b>M. Hallett, M.D.</b> <b>G. Elder, M.D.</b> <b>P. Plotz, M.D.</b> <b>G.H. Pezeshkpour, M.D.</b> <b>G. Di Chiro, M.D.</b> <b>G. Sanes, M.D.</b>	<b>Senior Staff Fellow</b> <b>Chief,</b> <b>Clinical Director,</b> <b>Neurologist</b> <b>Rheumatologist</b> <b>Neuropathologist</b> <b>Neuroradiologist</b> <b>Neurophysiologist</b>	<b>IDB, IRP, NINCDS</b> <b>IDB, IRP, NINCDS</b> <b>OCB, NINCDS</b> <b>IDB, IRP, NINCDS</b> <b>ARB, IRP, NIMSD</b> <b>AFIP, Wash. D.C.</b> <b>OD, IRP, NINCDS</b> <b>OCB, IRP, NINCDS</b>			
COOPERATING UNITS (if any) <b>AFIP, Washington, D.C.</b>					
LAB/BRANCH <b>Infectious Diseases Branch</b>					
SECTION <b>Immunochemistry and Clinical Investigations</b>					
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, Maryland 20892</b>					
TOTAL MAN-YEARS: <div style="text-align: center;"><b>1.5</b></div>	PROFESSIONAL: <div style="text-align: center;"><b>1.0</b></div>	OTHER: <div style="text-align: center;"><b>0.5</b></div>			
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews					
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Clinical and laboratory studies are conducted to determine etiology (infection, immunity and/or genetics) of chronic diseases of the peripheral and central nervous system. Current studies include amyotrophic lateral sclerosis, (ALS), polymyositis/dermatomyositis, new post-polio muscle weakness, demyelinating polyneuropathies, neuromuscular complications of AIDS and certain metabolic muscle diseases. Combined clinical data, genetic information, HLA and MLC typing and studies of virus serology and virus isolation are performed. A neuromuscular disease that occurs in patients who have had poliomyelitis at an early age has been clinically defined; the rate of progression of post-polio weakness was defined and its clinical electrophysiological, virological and histochemical similarities with ALS were explored. The nature of oligoclonal bands found in the CSF of patients with post-polio and other chronic neurological diseases is under investigation. Patients with polymyositis are studied and a viral mechanism is being investigated; the muscle changes in these patients before and after a randomized double-blind controlled study with cyclophosphamide, plasmapheresis or lymphocytapheresis are being investigated. The clinical, electrophysiological and immunological picture of a chronic, sensory, "ataxic" neuropathy were defined and the presence of a ganglionopathy was found; the pathogenetic mechanisms of this neuropathy were also studied and the role of proprioceptive afferent inputs for their postural maintenance was defined. The metabolic activity of the cortex in ALS patients was studied using the PET scan and <sup>18</sup>F 2-deoxy-D-glucose; hypometabolism was demonstrated not only in the motor but throughout the cortex, suggesting that ALS is a generalized process affecting many cortical regions. An experimental therapeutic trial with recombinant DNA alpha, human interferon was also in ALS patients. Muscle biopsies from patients with nephropathic cystinosis and renal Fanconi syndrome were studied morphologically and biochemically. Signs of a metabolic lipid storage myopathy due to carnitine deficiency were found; this prompted us to start a therapeutic study with carnitine replacement.</u> </p>					

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01-NS-01731-18-ID
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Isolation, Characterization and Diagnosis of Infectious Agents from Chronic Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	M. Gravell, Ph.D.      Research Microbiologist	IDB, IRP, NINCDS
Others:	R.S. Hamilton      Biologist	IDB, IRP, NINCDS
	M. Monzon, Ph.D.      Virologist	IDB, IRP, NINCDS
COOPERATING UNITS (if any) Section on Experimental Pathology, IDB, IRP, NINCDS		
LAB/BRANCH Infectious Diseases Branch		
SECTION Neurovirology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.1	.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <u>In vivo</u> and <u>in vitro</u> translation studies have shown that <u>genomic RNA</u> of <u>simian hemorrhagic fever (SHF) virus</u> codes for both <u>structural</u> and <u>nonstructural polypeptides</u> . Virions were shown to contain 3 structural polypeptides of approximately 50K, 18K and 12K, the largest of which is a glycoprotein. SHF virus RNA also was found to have a <u>type 1 cap</u> structure. In these characteristics, SHF virus has the replication strategy, number of type of polypeptides and cap structure of <u>Flaviviridae</u> , a recently formed virus family.		
19-IDB/IRP		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02602-03-ID

PERIOD COVERED  
October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Virus Induced Myelin Dysgenesis \*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B.J. Potts, Ph.D. Staff Fellow IDB, IRP, NINCDS

Others: G.A. Elder, M.D. Medical Staff Fellow IDB, IRP, NINCDS  
D. Huddleston Biologist IDB, IRP, NINCDS

COOPERATING UNITS (if any)

University of California Davis, Dept. Path., School of Vet. Med., Davis, CA;  
Developmental and Metabolic Neurology Branch, NINCDS  
University of Tennessee, Center Health Sciences, Memphis, TN

LAB/BRANCH  
Infectious Diseases Branch

SECTION  
Immunochemistry and Clinical Investigations Section

INSTITUTE AND LOCATION  
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	2.0	PROFESSIONAL:	1.5	OTHER:	.5
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Genetic disorders of myelin make it possible to study the steps in myelinogenesis. A new area important to these studies is that of virus induced hypomyelination. Congenitally acquired Border disease (BD) virus in sheep causes a selective disordering of myelin synthesis and provides another means of studying early steps in CNS myelinogenesis. This program focuses on studying events that regulate oligodendrocyte differentiation by the use of BD virus in congenitally infected fetal sheep CNS and in a differentiating neural cell culture system. Immunoglobulins are used to identify the major glial and viral antigens in the cell cultures, in CNS tissues and for use in identifying polypeptides in polyacrylamide gels. Immunocytochemical (ICC) studies are done with the light microscope (LM) and the electron microscope (EM). Antigens are co-localized by dual immune labeling using fluorochrome or colloidal gold labeled antibodies. The animal studies have determined that although BD virus infects many cell types in the CNS (granule cell neurons, oligodendrocytes and a rare astrocyte), it affects the oligodendrocyte at a critical stage in development causing a delay in myelinogenesis. In the acute infection at mid-gestation the earliest marker for oligodendrocytes, galactocerebroside (Gal-C), and a latter marker myelin basic protein (MBP), are absent. This reduction of myelin components persists in the chronic infection where a differential decrease in three myelin related proteins, myelin associated glycoprotein, MBP and proteolipid protein is noted. To determine how BD virus delays oligodendroglial maturation, a differentiating neural cell culture system was developed from fetal sheep CNS. Proliferating O-2A precursor glial cells expressing only A2B5 could be cultured under conditions in which they either remained O-2A cells with a characteristic bipolar morphology or began to express vimentin and GFAP in addition to A2B5 and acquired the morphology of astrocytes. The affects of BD virus replication on this cell system is under study.

\* { Formerly "Border Disease Virus: Structure, Replication and Pathogenesis" }  
21-IDB/IRP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01-NS-02531-05-ID			
PERIOD COVERED October 1, 1985 through September 30, 1986					
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Studies in Neuromuscular and CNS Diseases and Their Experimental Models</b>					
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;">           PI: M. C. Dalakas, M.D.            Others: J. L. Sever, M.D., Ph.D.            D. L. Madden, D.V.M., Ph.D.            M. Gravell, Ph.D.            W. T. London, D.V.M.            R. Quarles, Ph.D.            G.H. Pezeshkpour, M.D.         </td> <td style="width: 50%; vertical-align: top;">           Senior Staff Fellow            Chief            Veterinary Director            Research Microbiologist            Veterinary Director            Biochemist            Neuropathologist         </td> <td style="width: 50%; vertical-align: top;">           IDB, IRP, NINCDS            IDB, IRP, NINCDS            IDB, IRP, NINCDS            IDB, IRP, NINCDS            IDB, IRP, NINCDS            DMN, IRP, NINCDS            AFIP, Wash. D.C.         </td> </tr> </table>			PI: M. C. Dalakas, M.D. Others: J. L. Sever, M.D., Ph.D. D. L. Madden, D.V.M., Ph.D. M. Gravell, Ph.D. W. T. London, D.V.M. R. Quarles, Ph.D. G.H. Pezeshkpour, M.D.	Senior Staff Fellow Chief Veterinary Director Research Microbiologist Veterinary Director Biochemist Neuropathologist	IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS DMN, IRP, NINCDS AFIP, Wash. D.C.
PI: M. C. Dalakas, M.D. Others: J. L. Sever, M.D., Ph.D. D. L. Madden, D.V.M., Ph.D. M. Gravell, Ph.D. W. T. London, D.V.M. R. Quarles, Ph.D. G.H. Pezeshkpour, M.D.	Senior Staff Fellow Chief Veterinary Director Research Microbiologist Veterinary Director Biochemist Neuropathologist	IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS DMN, IRP, NINCDS AFIP, Wash. D.C.			
COOPERATING UNITS (if any)  AFIP, Washington, D.C					
LAB/BRANCH Infectious Diseases Branch					
SECTION Immunochemistry and Clinical Investigations					
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892					
TOTAL MAN-YEARS:  <div style="text-align: center;">1.5</div>	PROFESSIONAL:  <div style="text-align: center;">1.0</div>	OTHER:  <div style="text-align: center;">.5</div>			
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews					
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided ) <p> <u>Enzyme histochemistry in muscle and nerve biopsies</u> is carried out for diagnostic purposes in patients with several <u>neuromuscular disorders</u>. <u>Immunocytochemical studies</u> were conducted using specific antibodies to <u>thymic peptides</u> to investigate changes in the distribution of epithelial cells in the thymus of patients with <u>myasthenia gravis</u>. The interaction between cells of the <u>lymphoid and central nervous system</u> was investigated searching for common antigenic markers on their cell surface. <u>Thymosin beta<sub>4</sub></u>, an immunomodulating polypeptide, was found to be a common antigen shared by macrophages, dendritic lymphoid cells and oligodendrocytes. The IgM of certain patients with <u>paraproteinemic polyneuropathies</u> has been identified as a specific antibody to <u>myelin associated glycoprotein</u> or glycolipids; <u>nerve biopsies</u> from these patients are studied by <u>electron microscopy</u> and <u>immunocytochemically</u>. The nature of <u>amyloid protein</u> in patients with "sporadic" <u>amyloid polyneuropathy</u> was identified using specific antibodies to amyloid proteins immunocytochemically and biochemically on the <u>extracted amyloid</u>. <u>Immune cellular markers</u> were investigated during the evolution of <u>EAN and EAE</u> induced in rhesus monkeys and <u>therapies</u> were attempted using some novel <u>immunomodulating agents</u>. The mechanism of <u>inflammatory myopathy</u> in monkeys with <u>immunodeficiency (Simian AIDS)</u> due to a retrovirus D, (SRV-1), was further studied. Antibodies to SRV-1 immunoreacted with the <u>inflammatory cells invading the muscle fibers</u>; SRV-1 was capable of infecting <u>myoblasts</u> in tissue culture without exerting a cytopathic effect in the muscle. <u>Polymyositis and inflammatory neuropathies</u> were also identified in patients with <u>AIDS</u> and the mechanism of muscle or nerve damage is being investigated with <u>in situ</u> hybridization and immunocytochemistry. The effect of <u>aging on the neuromuscular system</u> of monkeys from age 5 to 25 is being investigated with a detailed morphological and morphometrical analysis of their muscle and nerve biopsies. Morphological changes in the muscles of <u>autoimmune mice</u> are also studied; <u>tubular aggregates</u> were found and their relation to <u>endogenous interferon</u> is being investigated.         </p>					

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-NS-00972-15-ID

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Models for CNS Infections in Normal and Immunocompromised Hosts

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	W.T. London, D.V.M.	Veterinary Director	IDB, IRP, NINCDS
Others:	M. Gravel, Ph.D.	Research Microbiologist	IDB, IRP, NINCDS
	V.G. Hemming, M.D.	Associate Professor	USUHS
	J.L. Sever, M.D., Ph.D.	Chief	IDB, IRP, NINCDS
	S.A. Houff, M.D.	Neurologist	IDB, IRP, NINCDS
	M.C. Dalakas, M.D.	Senior Staff Fellow	IDB, IRP, NINCDS
	A.J. Martinez, M.D.	Prof. Neuropathology	U PITT SCH MED
	J.M. Phillips, D.V.M.	Dir. Animal Medicine	MELOY LAB.
	B.L. Curfman	Biologist	IDB, IRP, NINCDS
	R.L. Brown	Biological Lab Technician	IDB, IRP, NINCDS

## COOPERATING UNITS (if any)

Uniformed Services University of the Health Sciences, Bethesda, Maryland;  
 University of Pittsburgh Presbyterian Hospital, Department of Neuropathology,  
 Pittsburgh, Pennsylvania; Meloy Laboratories, Inc., Springfield, Virginia

## LAB/BRANCH

Infectious Diseases Branch

## SECTION

Experimental Pathology Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.70

## PROFESSIONAL:

.60

## OTHER:

2.10

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Rhesus Cytomegalovirus (RCMV). Severe congenital infection and disease are produced in rhesus fetuses inoculated intraamniotically or intracerebrally with RCMV. Lesions confined to the CNS consisted of ventricular dilatation, leptomenigitis choroid plexitis, ependymitis and focal encephalitis. Ventricular dilatation appears to be the result of severe tissue destruction of the subependymal area since ventricular obstruction is not present. This model will be useful in the study of the pathogenesis of congenital CMV infections.

Group B Streptococci (GBS). Maternal immunization may not be the best means to protect the fetus. We found maternal humoral immunity insufficient to prevent GBS infection of the rhesus monkey fetus. Therefore, treatment of the newborn infant with hyperimmune human IgG was studied. Results indicate that severe GBS infection in the rhesus newborn can be treated successfully with hyperimmune immunoglobulin. Our findings have since been used to justify the treatment of infant humans infected with GBS.

SAIDS. Polymyositis developed in 50% of the rhesus monkeys that developed clinical signs of SAIDS. The morphologic features of polymyositis in the monkey are identical to those seen in human polymyositis. We suspect a possible association between a human retrovirus and some cases of polymyositis in man.

Varicella-Zoster. A new strain of simian varicella-zoster virus has been isolated from a rhesus monkey (Macaca mulatta) with a typical chickenpox-like illness. This new strain of virus has potential use for establishing an animal model of human varicella-zoster.

23-IDB/IRP









# ANNUAL REPORT

October 1, 1985 through September 30, 1986

## Medical Neurology Branch, IRP

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT  
October 1, 1985 through September 30, 1986

Medical Neurology Branch, IRP

National Institute of Neurological and Communicative Disorders and Stroke

Chief, Roger J. Porter, M.D.

The Medical Neurology Branch conducts research on human epilepsy, including new approaches to diagnosis and treatment, investigates basic questions related to normal and abnormal neuronal excitability, performs studies on human motor control and speech, conducts research on Alzheimer's disease and related disorders including autonomic dysfunction, and investigates cognitive and emotional processes in man.

The Branch is divided into five Sections. Roger J. Porter, M.D., is Chief of the Clinical Epilepsy Section; Mark Hallett, M.D., Chief of the Human Motor Control Section; Ronald J. Polinsky, M.D., Chief of the Clinical Neuropharmacology Section; and Paul Fedio, Jr., Ph.D., Chief of the Clinical Neuropsychology Section. Dr. Porter serves as Acting Chief of the Neuronal Excitability Section.

CLINICAL EPILEPSY SECTION

The Clinical Epilepsy Section is undertaking a series of studies using new techniques in order to improve clinical control in patients with refractory seizure problems, as well as to elucidate the pathophysiology of epilepsy. Emphasis is being placed on positron emission tomography (PET) as a technique to investigate basic mechanisms of cerebral metabolism in epilepsy and to assist in the clinical evaluation of patients with severe partial seizures. Ultrastructural and biochemical investigations of epileptic tissue removed at surgery will be correlated with metabolic findings. Studies have begun with magnetic resonance imaging (MRI), which has potential for more precise localization of epileptogenic lesions, as well as for elucidation of the anatomical substrates of altered physiologic patterns revealed by PET.

Patients with severe uncontrolled seizures are admitted to the Clinical Center according to the following criteria: 1) patients with complex partial seizures, especially those who may be candidates for PET scan evaluation and surgical therapy; and 2) patients with absence seizures or atonic/myoclonic for studies of cerebral metabolism including the effect of antiepileptic drugs. After seizure frequency and type is characterized by intensive monitoring techniques, the patients are placed in an appropriate research protocol. After the research protocol is completed, each patient's therapeutic regimen is adjusted to obtain optimal seizure control.

PET is a technique using the intravenous injection of a radioactive isotope to determine regional rates of cerebral metabolism. The Clinical Epilepsy Section has been using ( $^{18}\text{F}$ )-fluorodeoxyglucose (FDG) to measure the

regional cerebral use of glucose over a 30-minute period after the injection of isotope. Ongoing studies include (1) patterns of cerebral metabolism in patients with partial, generalized, and atonic/myoclonic seizures; (2) the effect of antiepileptic drugs on cerebral metabolism; (3) effect of chemical seizure activation on glucose metabolism; (4) correlation of neuropsychological tests with PET results; activation of normal tissue by a sustained language task; (5)  $^{15}\text{O}$  water will be used to study cerebral blood flow; (6) using a suitable ligand, benzodiazepine receptors will be studied in patients with epilepsy.

The role of MRI scanning in the seizure disorders is also being investigated. MRI shows more detailed anatomic images than CT and may detect subtle changes in cerebral density resulting from small gliotic regions which may be epileptogenic.

A specific protocol has also been designed to investigate the etiologies of selected patients with epilepsy and progressive neurologic deterioration. This study, which involves a multidisciplinary team of investigators, is capable of analyzing neurologic, electroencephalographic, radiologic, pathologic (including brain biopsy when indicated), metabolic, and virologic data in an attempt to delineate some of the causes of seizure disorders.

A project has been initiated to use sphenoidal, and in some cases subdural electrodes, in the evaluation of potential surgical candidates, coupled with long-term video-EEG recording techniques. These techniques allow the acquisition of EEG data not available via surface recordings. This data is correlated with PET and MRI to obtain the best possible presurgical localization of epileptic foci. A prospective evaluation of the relative value of invasive (subdural) and noninvasive methods of presurgical evaluation has begun.

During surgery, direct electrocorticographic recordings are made before and after tissue is resected, in order to guide the surgical approach. Tissue from both epileptogenic and nonepileptogenic regions is obtained for biochemical study.

Magnetoencephalography (MEG) is a new approach to the problem of localizing abnormal cerebral potentials which may represent an epileptic focus. Initial studies suggest that MEG may provide more precise three-dimensional information than EEG, allowing detection and localization of epileptic foci in the depths of the brain, without the need for invasive procedures.

Biochemical parameters of CSF in patients with epilepsy will be compared before and after seizures, and on changing drug regimens. Compounds to be studied include catecholamines, neuropeptides, and amino acids.

Pharmacologic studies in epilepsy continue to concentrate on studies of drug interactions and of new antiepileptic drugs. Patients with uncontrolled seizures, especially complex partial seizures or absence seizures, are accepted for study. Such patients usually have a detailed

seizure calendar available prior to entering the study; they enter a week-long period of baseline determination of seizure frequency and blood levels of antiepileptic drugs while in the hospital. Each pharmacologic protocol varies, but all require modification of the antiepileptic regimen and addition of the medication under study. This may be done in single dose or chronic administration studies depending upon the particular protocol in question. Plasma levels are often drawn daily, and on occasion, much more frequently for specific studies. Following the completion of the pharmacologic protocol, the patient is placed on a regimen which is best suited for the seizure type which has been identified by videotape/telemetered EEG analysis. This regimen is stabilized prior to discharge of the patient.

The Clinical Epilepsy Section has begun studies on a promising antiepileptic drug developed by Wallace Laboratories. The drug known as felbamate is 2-phenyl-1,3-propanediol dicarbamate. In pre-clinical testing the drug is effective in animal models which correlate with partial seizures in man and is quite nontoxic in animals. Evaluation of the protective indices indicate that felbamate has a significant and adequate margin of safety. In anticipation of a randomized placebo-controlled double-blind study, two pilot patients with partial seizures were studied. These pilot patients were performed in a single-blind fashion to observe for side effects of the medication as part of the dose ranging studies necessary prior to the double-blind study itself. Both of these patients tolerated felbamate very well; however, a dosing regimen change was introduced to decrease gastrointestinal side effects. Both of these patients have remained on felbamate, and they felt an improvement in seizure control. Following the completion of the pilot patients the randomized placebo-controlled double-blind study was initiated. The purpose of this study is to obtain definitive information regarding the efficacy and safety of felbamate in patients with uncontrolled partial seizures who are receiving concomitant carbamazepine therapy.

In a separate study of patients with partial seizures, a pilot effort will be undertaken to evaluate a very old compound, dapson, for its effect on control of partial seizures. This drug has been used for decades in the treatment of leprosy as well as in dermatologic disorders; more recently it has demonstrated efficacy in animal models--such as the maximal electroshock--and its protective index is promising. This pilot study will not be a controlled clinical trial but will evaluate the pharmacology of the drug in humans, the drug interactions with current antiepileptic drugs, and will obtain some preliminary assessment of efficacy of the drug.

Studies are now complete on our most recent evaluation of phenytoin, one of the most commonly used antiepileptic drugs. On study of the plasma phenytoin fluctuations after dosage change, we observed that saturation kinetics may only predict a part of what we have termed the pseudosteady-state phenomena and that a multi-compartment kinetic model with prolonged distribution equilibrium may be necessary to account for the experimental observations.

Recent studies have also been completed on the evaluation of free rather than total phenytoin levels. In a study of 80 patients it was demonstrated there is little obvious superiority of free over total levels in predicting drug toxicity or patient seizure frequency. In general therefore there is little clinical rationale for measuring free rather than total levels in patients without significant renal or hepatic dysfunction.

Still underway is a study evaluating the relative potencies of a widely used antiepileptic drug, carbamazepine, and its metabolites, especially carbamazepine 10-11 epoxide. This study, which evaluates both toxicity and seizure control, may prove valuable in designing optimal antiepileptic drug treatment regimens because the role of the metabolite will be better understood.

## HUMAN MOTOR CONTROL SECTION

The mission of the Section is to understand principles of normal motor control in man and the pathophysiology of motor disorders including both deranged voluntary movement and involuntary movement. The Section is composed of a Speech Pathology Unit and a Movement Disorders Unit.

### Speech Pathology Unit

The goal of the Speech Pathology Unit is to determine the neurological organization of speech and phonatory control through the study of the breakdown of these functions in neurological disorders. The research continues to address three major issues: 1) The neurophysiological bases of phonatory disorders; 2) Speech production timing as evidenced in neuropathologies of speech; and 3) The brain organization of speech production and speech perception from brain lesion studies.

**Phonatory Function:** To determine the pathophysiology of spasmodic dysphonia, neurophysiological techniques were developed for recording with concentric needles from two intrinsic laryngeal muscles, the thyroarytenoid and cricothyroid. A new laboratory computer system was developed and the signal processing programs completed. The first study completed this year compared laryngeal muscle activation patterns during quiet respiration and phonation in spasmodic dysphonia and normal controls. Maximum levels of muscle recruitment prior to, at onset and during phonation were higher in the patients. However, the percent increase in activation over resting levels were the same in the patients as the controls for the thyroarytenoid muscles. Therefore, increased muscle tone was present both at rest and during phonation in the patients. Further, the activation ratios of the cricothyroid muscles were higher than in the thyroarytenoid muscles in the controls but not in the patients. Thus, muscle recruitment levels and patterns of activation between the laryngeal muscles differed between the two groups.

To determine whether spasmodic dysphonia is a task specific focal dystonia, muscle activation patterns of the two groups were also compared during quiet respiration. Both minimum and maximum activation levels during respiration were significantly greater in the patients. Therefore,



laryngeal muscle tone and activation levels were increased in spasmodic dysphonia over normal in other tasks besides phonation. A temporary unilateral recurrent laryngeal nerve block was used to determine whether muscle activation patterns on the opposite side of the larynx are reduced with a reduction in muscle spindle feedback. In the first four patients, activation was reduced during respiration on the opposite side following the nerve block. However, during phonation activation increased on the side opposite the block possibly due to the need to overadduct the unaffected side to achieve vocal fold closure for phonation. Additional patients have been tested and their data is being analyzed.

Testing is ongoing on two additional studies. In one, to determine the effects of a central depressant on muscle activation levels in spasmodic dysphonia, the effects of diazepam on muscle activation patterns in patients and controls is being determined both at rest and during phonation. Thus far, the data suggest a depressive effect on laryngeal muscle activation in normals and not in patients. In another, to determine whether recurrent laryngeal nerve surgery alters the pathophysiology of spasmodic dysphonia, the activation levels on the unoperated side of patients who have had unilateral recurrent nerve sections are being compared with those of spasmodic dysphonic patients who have not had surgery. Reinnervation has been found in all four post surgical patients studied thus far, with reduced activation levels on the unoperated side. These studies suggest reduced central inhibition of laryngeal muscle activation in spasmodic dysphonia with increased sensitivity to muscle spindle feedback. Studies examining the effects of electrical and auditory stimulation on muscle activation are planned to further examine the pathophysiology of this disorder.

Since botulinum toxin injections have been successful in treatment of other focal dystonias such as blepharospasm, two trials of botulinum toxin injections for the temporary alleviation of phonatory spasms were conducted this year. In the first trial, single 10 Units/.1 ml injections into the left thyroarytenoid were administered and followed two weeks later by 10 Units into both the left thyroarytenoid and cricothyroid muscles. This was repeated again 2 weeks later if no effect on vocal fold movement was seen. Three patients participated; two who previously had surgery of the left recurrent laryngeal nerve were injected on that side and one with no previous surgery. One patient, the mildest, had a response after the second injection. Vocal fold movement on the injected side was reduced within 2 days following injection and the voice remained aphonic for 5 days. With gradual voice return, few breaks occurred in phonation for 35 days with symptoms returning within 45 days.

In the second trial, 10 Units of toxin were injected at reduced concentration (5 Units/.1 ml.) in 6 different sites within the left thyroarytenoid muscle on the first injection and 15 Units on second injection, 2 weeks later. Five of the 6 patients had reduced vocal fold movement and a change in voice 2 days following the second injection. Only the most severe patient did not have a response with no change in vocal fold movement on the injected side. In the patients with a beneficial

response, the voice first became breathy but returned within 7 days with reduced phonatory breaks. This improvement remained for 4-5 weeks with symptoms gradually returning in the 6th week. The patient's report continued ease of phonation into the 7th week. The only negative side effect has been some swelling in the laryngeal area interfering with movement speed during swallowing of liquids. This starts within 3 days after the second injection and can continue up to 7 days. Laryngeal electromyography is being conducted pre- and post-treatment in these patients to determine whether this treatment alters the pathophysiology seen in spasmodic dysphonia. Objective methods of quantifying phonatory function in spasmodic dysphonia over time were developed and will be used to evaluate patients pre- and post- botulinum treatment.

**Speech Timing:** An acoustic study of speech in Parkinson's disease patients' speech completed this year found no difficulties with place of articulation contrasts in a controlled experimental setting, although their speech was perceptually impaired when not experimentally controlled. A new series of studies were initiated this year as an outgrowth of this research and of our previous acoustic studies. The purpose is to examine the effects of linguistic frequency, length, and preplanning on speech production in both normal adults and patients with Parkinson's disease. The timing of speech initiation, movement velocity, muscle activation patterns and articulator coordination for respiratory, lip, jaw and laryngeal movement are being measured to determine the effects of different linguistic encoding demands on speech production. It is hypothesized that patients with dysarthria associated with neurological disease are most affected in the enactment of speech movements in complex and unfamiliar utterances requiring online planning and organization. This might explain their poor speech intelligibility in conversational settings in contrast with controlled testing situations when their speech skills are close to normal.

**Neurological Organization of Speech and Language:** Analyses of speech and language data from the Vietnam Head Injury Study are continuing to address the neurological organization and inter-relationships between speech production, speech perception and language. These projects are aimed at determining the lesion topography of penetrating missile wounds underlying specific chronic deficit patterns 15 years post injury. Two subtypes of persistent speech dyspraxia were studied; one with associated nonfluent aphasia, the other without any language deficits. When contrasted with other penetrating brain lesion locations in patients without speech/language deficits, all speech dyspraxia patients had left cortical involvement of the primary motor strip (area 4), partial extension to areas 6, 8 and 9 and deep extension to the insula, superior longitudinal fasciculus, frontal corona radiata and the anterior corpus callosum. Experimental speech testing demonstrated that despite large lesions involving the pyramidal tract and cortical regions associated with speech production in the left hemisphere, some patients had only mild chronic residual speech deficits suggesting considerable reorganization for speech motor control following penetrating missile wounds in young men.

#### Movement Disorders Unit

The Movement Disorders Unit has now been active for about one year. A number of projects are in progress.

Studies using the Gait Laboratory of the Department of Rehabilitation Medicine have focused on the control of balance. Simultaneous measurement of body angles, foot-floor forces and multiple EMG's are possible. Studies in normal subjects have revealed insights into the biomechanical effects of postural muscle activity. Similar studies have been initiated in patients with cerebellar disturbance and Parkinson's Disease. Two patients with orthostatic tremor have also been investigated.

In patients with Parkinson's Disease there was a positive correlation between circulating levels of dopamine and cognitive motor capabilities in a choice reaction time situation but not in a simple reaction time situation. In normal subjects, triggered muscle responses produced by stopping voluntary movements were similar to muscle responses generated when subjects initiated a voluntary movement from the stopped location, thereby suggesting that triggered and voluntary reactions are mediated by similar central nervous system mechanisms.

The study of motor control in hemiplegia is being planned, and apparatus is being prepared. Patients with discrete brain lesions will be studied; patients with strokes will be the main group, and many will be followed serially from onset of the disorder to recovery. Preliminary studies with positron emission tomography (PET) of normal subjects have shown that it is difficult to identify cortical areas active with voluntary movement using ( $^{18}$ F)-fluorodeoxyglucose (FDG) as the tracer. Further studies are planned with oxygen-15 water which will probe blood flow rather than glucose metabolism.

Myoclonus and a number of other rapid involuntary movements have been difficult to classify clinically. Clinical and physiological analysis of a continuing series of patients has led to new classifications and pathophysiological insights. A series of patients with epilepsia partialis continua have been studied; the pathophysiology is heterogeneous, but, in most, electrophysiological techniques can identify an excitable region in cerebral cortex. A series of patients with adult onset tic have been analyzed and clinical rules have been formulated to make this diagnosis. Positron emission tomography (PET) studies of patients with palatal myoclonus have revealed that the inferior olives are hyperactive. PET studies of patients with hemiballismus have revealed that hypoactivity of the ipsilateral striatum occurs as a result of the lesion in the subthalamic nucleus.

In studies of postural action tremors, we have been analyzing the amplitude of the tremor as a function of the precise posture of the limbs. For many tremors, such as cerebellar postural tremor, the tremor is worse when the arms are near the body, and the hands are pointing towards each other. For other tremors, this is not true, and the clinical and physiological significance of this is being explored.

Task specific focal dystonias of the hands, such as writer's cramp and

pianist's cramp, have been analyzed, and a number of physiological characteristics have been defined. There appears to be diminished ability to control the fingers independently, and gating of somatosensory evoked potentials with voluntary movement is abnormal. The spasms themselves have been characterized into different patterns. Abnormalities of the blink reflex have been identified in dystonic disorders. We have verified this in a number of our own patients, and are now applying this test to the patients with focal hand cramps.

Recently techniques have become available for the non-invasive stimulation of the human cortex and human spinal cord. A device has been manufactured which produces a high voltage, extremely brief pulse which can penetrate skull and activate central nervous system without excessive pain. Previous studies have shown it to be useful in some circumstances where evaluation of the speed and integrity of motor pathways is valuable.

We have just begun to use this technique. Our first project is to see if we can map the motor cortex.

Previous studies have shown utility of isoniazid for ameliorating cerebellar postural tremor in patients with multiple sclerosis. Current studies are aimed at identifying whether patients with action tremors of other types are also benefitted. A double-blind placebo-controlled, cross-over trial is in progress. Thirteen patients have now been studied. Isoniazid does appear to help some of these cases, but we do not have enough experience yet to predict the response.

Botulinum toxin injected in small doses directly into muscle binds to the neuromuscular junction, and inactivates it for approximately 3 months. This treatment has been demonstrated to be useful for strabismus and blepharospasm, but there has not been a complete understanding of its mechanism of action.

Studies of utility of botulinum toxin have been carried out in spasmodic dysphonia and writer's cramp (and its variants, such as pianist's cramp). Treatment appears efficacious for both. Further studies are planned with adult stuttering.

Studies of physiology of the mode of action have been carried out in spasmodic dysphonia, writer's cramp, blepharospasm and hemifacial spasm. These studies are currently incomplete.

## CLINICAL NEUROPHARMACOLOGY SECTION

The Clinical Neuropharmacology Section continues to develop clinical, physiological, biochemical and pharmacological methods for assessment of autonomic nervous system function in man. Since norepinephrine is the neurotransmitter released by most post-ganglionic sympathetic nerve endings and is also an important central nervous system neurotransmitter, these

investigations have focused primarily on the noradrenergic system. High performance liquid chromatography, liquid scintillation spectrometry, and mass spectroscopy are used to measure neurotransmitter and metabolite levels in plasma, urine, and cerebrospinal fluid under basal conditions and after a variety of stimuli have been applied to elicit a sympathetic response.

Peptide and hormonal levels are determined by radioimmunoassay. Autonomic failure may occur alone (idiopathic orthostatic hypotension, IOH) or in association with a central nervous system degeneration (multiple system atrophy, MSA). Investigation of patients with lesion(s) of the autonomic nervous system has provided an opportunity to examine the interaction between the autonomic nervous system and other hormonal/peptide systems.

We have continued to investigate insulin-induced hypotension since endogenous insulin release may play an important role in causing post-prandial hypotension. The mechanism of insulin-induced hypotension may differ between IOH and MSA. Our previous studies showed that it was not due to excessive b-adrenergic stimulation. Although patients with MSA or IOH may have deficient catecholamine responses to insulin-induced hypoglycemia, only MSA patients have diminished b-endorphin responses. Thus, the hypotensive effect of b-endorphin released during hypoglycemia may be unopposed due to an inadequate norepinephrine response in IOH patients. In MSA, the hypotension might result from direct stimulation of insulin receptors present in the brain. The consequences of hypoglycemia have been separated from the other effects of insulin by using the glucose-clamp technique. Glucose infusion prevents the drop in blood pressure following insulin administration in MSA but not IOH patients. Preliminary results in two patients (1 MSA, 1 IOH) indicate that naloxone did not significantly reduce the fall in blood pressure during an insulin tolerance test. Additional studies will utilize venous plethysmography measurements of peripheral vascular resistance and venous compliance to further elucidate the cardiovascular aspects of this abnormal clinical response. Measurements of other peptides are also in progress to determine whether the hypothalamo-pituitary involvement in MSA is selective for ACTH and b-endorphin. Hypothalamic degeneration in MSA is attended by a reduction in choline acetyltransferase, a marker of cholinergic neuronal integrity. Arecoline, a muscarinic cholinergic agonist, will be used to assess the integrity of the cholinergic pathway which is involved in the control of b-endorphin release during hypoglycemia.

Several strategies have been applied to examine brain structure and function in MSA. Magnetic resonance imaging reveals a reduction of signal intensity in the putamen of patients with MSA which correlates with the

clinical severity of parkinsonism and is consistent with the known neuropathology. Marked atrophy in the posterior fossa and brainstem was observed in those patients with the olivopontocerebellar form of the disease. These abnormalities were not observed in a preliminary analysis of the MRI scans in 4 patients with IOH. Cerebral glucose metabolism, measured by positron emission tomography, also appears to be normal in IOH.

A more detailed analysis of the results is necessary to understand the heterogeneous results obtained in the series of MSA cases. Neuropathological examination in four of our MSA cases reveals extensive cell loss and gliosis in the putamen, globus pallidus, cerebellum, brainstem and spinal cord. Detailed clinicopathological correlations and studies of regional neurochemistry are planned.

Our previous study revealed a decrease in the plasma disappearance rate of radiolabelled norepinephrine after infusion to steady-state levels in IOH patients. This is a reflection of diminished neuronal uptake of norepinephrine. However, the stereospecific labelling pattern of urinary norepinephrine metabolites was not significantly different among control subjects, IOH and MSA patients. In normal subjects, most vanillylmandelic acid (VMA) is derived from 3-methoxy-4-hydroxyphenylglycol (MHPG). Approximately two-thirds of normetanephrine is also converted into VMA. Methods are currently being developed to measure red blood cell norepinephrine in order to examine the ratios of radiolabelled catecholamine isomers taken up during the steady-state infusions. The red blood cell may serve as a model for studying neuronal uptake.

Biochemical and pharmacological investigation of autonomic nervous system function has been completed in 5 family members with hereditary adult-onset leukodystrophy. These patients have orthostatic hypotension as well as other signs of autonomic dysfunction. Low supine norepinephrine levels and a parallel shift to the left of the blood pressure dose-response curve to norepinephrine imply that the orthostatic hypotension results from a lesion confined to the peripheral sympathetic nervous system. This disorder is also attended by adrenal medullary involvement as demonstrated by the deficient epinephrine response to insulin-induced hypoglycemia.

Studies of neurotransmitter metabolism have been completed in other patient groups, but the data analysis is only at a preliminary stage. Elevation of basal plasma norepinephrine levels in narcolepsy patients is consistent with overactivity of the sympathetic nervous system. Cerebrospinal fluid levels of MHPG, homovanillic acid, and 5-hydroxyindoleacetic acid are normal in adult patients with Gilles de la Tourette syndrome. These results do not support a neurochemical basis for the disease involving noradrenergic, dopaminergic, or serotonergic systems.

A number of additional studies are currently in progress:

- 1) Cardiovascular and catecholamine responses to intravenously administered acetylcholine are being studied in patients with MSA and IOH. The purpose of these studies is to look for evidence of ganglionic supersensitivity which may differ according to the site of the autonomic nervous system lesion.
- 2) Studies of autonomic function in narcolepsy and aging have continued. Additional subjects must participate before meaningful results can be obtained.

- 3) Data collection has been completed for the clinical and family studies involving 55 subjects with progressive autonomic failure. There does not appear to be an hereditary pattern of either MSA or IOH. A more complete analysis of many aspects of clinical expression, associated disorders, and other factors is in progress. An HLA association study in IOH and MSA patients is also being conducted.
- 4) A collaborative investigation to detect antibodies to nicotinic receptors in IOH is in progress.

The Clinical Neuropharmacology Section has continued the study of familial Alzheimer's disease (AD) as a major priority within the scope of its research efforts. Alzheimer's disease is a major medical and social problem since it is the most common cause of irreversible, chronic dementia. Clinical and therapeutic research of AD is significantly limited by both accuracy and timing of diagnosis. More than 30% of clinically diagnosed cases do not have AD at autopsy. Although AD may be familial in only about a third of all cases, the main justification for studying the autosomal dominant subgroup of familial cases lies in the accuracy of diagnosis which may be inferred through post-mortem examination of other affected family members. There are two major directions of our AD research: (1) to investigate genetic linkage in order to identify the primary molecular event underlying AD in these families, and (2) to define the clinical and biochemical progression of the disease through a longitudinal investigation of affected and at-risk subjects. The ultimate goal of our work is to provide clues for earlier, accurate diagnosis and for more rational approaches to treatment of AD.

The intra-agency agreement between the NINCDS and NIA has facilitated the establishment of skin fibroblast and peripheral blood lymphoblast cultures at the Cornell Institute for Medical Research, Camden, New Jersey. Additional affected members as well as new branches have been identified in the large AD pedigrees with autosomal dominant inheritance. More than 125 individuals from 5 pedigrees (2 U.S., 1 Italian, 1 German, 1 Canadian) have been examined and biopsied. From the clinical standpoint, there appears to be a well-defined pattern of disease expression within families but there is heterogeneity among families. A more detailed analysis of the clinical, genetic, and neurological aspects of disease expression is in progress. The cultures which have been established continue to serve as a renewable source of DNA and cells for basic research in AD.

Genetic linkage studies using restriction fragment length polymorphisms recognized by suitable DNA probes have focused on chromosome 21. Using a multi-point linkage analysis in three of the large kindreds, it has been possible to exclude approximately 70% of chromosome 21. Probes are being developed to complete the study of this chromosome. Based on studies of classical genetic markers (specifically HLA and Gm), previous studies suggested the importance of chromosome 14 in the etiology of inherited AD. However, linkage has now been excluded within the region of 5 centimorgans on either side of probe D14S1 which includes the Gm locus.

A partial cDNA which codes for the 200 kilodalton neurofilament peptide (NF 200) has been cloned; six different polymorphisms have been identified in the Canadian pedigree and linkage studies are in progress. A number of other single candidate genes are being studied, e.g. somatostatin, DNA repair gene, scrapie protein cDNAs, acetylcholinesterase, and small neurofilament peptides.

Studies of DNA repair in familial AD lymphoblastoid lines have continued using alkaline elution to measure changes in DNA size after exposure to DNA-damaging agents. Although differences were observed in a pilot study of 12 cell lines exposed to methyl methane sulfonate, the changes did not segregate according to diagnosis. However, a clear difference between affected and control lines occurred using N<sup>1</sup>-methyl-N-nitro-N-nitrosoguanidine. Of two at-risk lines studied, one was normal and the other had a low level of repair. Additional lines are currently under investigation. Clarification of the repair defect will be accomplished by development of a cloning assay, use of longer recovery times, analysis of dose-response curves, and examination of enzymatic aspects of DNA repair.

The longitudinal investigation has continued and psychological support and genetic counseling have been offered. Although preliminary evidence suggests that noradrenergic involvement may occur early in AD, it is necessary to study more family members in order to make a definitive statement. However, the results of an initial study of brain glucose metabolism have been analyzed. Global and regional rates for cerebral glucose metabolism (CGMR) were measured by positron emission tomography in two patients and two asymptomatic at-risk subjects from families with histologically confirmed, dominantly inherited Alzheimer's disease (AD). In the AD patients, there was a bilateral, symmetrical reduction in CGMR of approximately 35% compared to age-matched control subjects. The supramarginal gyri and temporal lobes were selectively involved out of proportion to other brain regions. A complete region of interest analysis revealed an isolated, significant reduction of CGMR in the left supramarginal gyrus of one at-risk subject. The pattern of hypometabolism encountered in our patients with dominantly inherited AD is similar to that reported for the sporadic form of the disease. Although the CGMR reduction in the at-risk subject occurred in an area affected in the AD patients, additional follow-up studies will be necessary to determine whether changes in CGMR precede clinical expression of the disease.

The clinical and family study of 22 twin pairs with one or both having dementia of the Alzheimer type (DAT) was completed. Seven monozygotic (MZ) pairs were concordant for DAT; ten MZ pairs were discordant; two dizygotic (DZ) pairs were concordant and three DZ pairs were discordant. The current concordance rate was 41% for MZ twins and 40% for DZ twins. The age of onset in concordant twins varied as much as ten years. The study supports the belief that etiologically, DAT cannot be entirely accounted for by a single autosomal dominant gene. The data also suggested that in certain genetic circumstances, disease expression may be delayed in females.

Neuropathological examination of specimens from the families with



dominantly inherited AD have concentrated on the hippocampus. Approximately half of the cases display pathological changes in Sommer's sector. There is a marked variability of the autopsied cases within the Canadian pedigree. Three of the four cases studied have senile plaques and tangles with plaques being the striking feature. The remaining case showed abundant neurofibrillary tangles while senile plaques were rare in the hippocampus but abundant in the neocortex. No conclusive evidence of AD was found in the brain of a 48 year old asymptomatic at-risk family member who died from a malignant melanoma.

#### CLINICAL NEUROPSYCHOLOGY SECTION

Cognitive and emotional activities in man are dependent upon the integrity of the limbic system, and therefore, insult to this brain region affords neuroscientists an unique opportunity to examine and to better understand the neural basis of human behavior. In a series of neuropsychological studies, memory and perceptual mechanisms were evaluated in epileptic patients following a unilateral left or right temporal lobectomy. Using rapid delivery, tachistoscopic projection, right temporal patients required a longer exposure duration to detect the presence of a stimulus, but not to discriminate two versus single flashes. Left temporal patients, in contrast, exhibited the reverse pattern. These data suggest that right hemisphere mechanisms are optimally suited for summation of sensory input over time to yield heightened perceptual sensitivity, but at the expense of fine temporal resolution. Left temporal systems are better organized and suited to deal with fine temporal acuity, but at the expense of overall perceptual sensitivity.

There appears to be an asymmetry in the organization and expression of emotionality by the left and right limbic systems. For example, left temporal lobectomy patients expressed neutral ratings about scenic material that were ranked as pleasant or horrific by normal subjects. Patients with left removal also applied inappropriate verbal descriptions for visually presented sequences of emotional behaviors. The same response bias held for these patients in judging photographs of faces displaying different emotional expressions. There was less disruption by a right temporal resection. Moreover, the left and right temporal patients were physiologically unresponsive while viewing affective material, as indexed by skin conductance indices. Unlike normal subjects, the temporal lobectomy patients were unable to take advantage of the emotional coloration of information in facilitating subsequent recall.

Relatedly, the left and right temporal patients evaluated their behavior differently on a behavioral inventory. In comparison with nonoperative epileptic subjects, most patients acknowledged an improvement in their behavior following unilateral temporal lobectomy. Nonetheless, within the context of this general improvement, specific personality styles persisted and were dependent on the side of removal: the left temporal lobectomy patients viewed themselves as ideative, reflective and non-emotional, but

were overly harsh and self-critical in their ratings; in contrast, the right temporal patients regarded themselves as emotive, but in a more socially favorable light than their raters.

In an effort to assess the compensatory value of mnemonic strategies, temporal lobectomy patients were instructed in the use of different encoding cues to deal with postoperative memory difficulties. The study confirmed the facilitatory value of visual imagery by the neurosurgical patients, and matched groups of medical neurological patients and normal individuals. Abstract, low-imagery words were poorly recalled by the left temporal patients. In a separate study, subjects received instructions to either print a word or to sketch a picture of an object represented by a word, overtly (graphically) or covertly ('in mind's eye'); all groups remembered fewer printed than sketched words, and the left temporal group did less well in remembering sketched material. In another mnemonic paradigm utilizing phonetic, spatial, or praxic cues, all groups, particularly the left, did very poorly with phonetic encoding. In contrast, spatial and praxic mnemotechnics proved beneficial, more so for the left temporal patients. These data confirm that left temporal mechanisms are indispensable to encode verbal information during initial learning. Modest compensation for memory defects following temporal lobectomy may be achieved with strategies that combine overt or covert imagery with praxic encoding.

Extending these behavioral changes with computer derived, electrophysiological indices (P300 events), procedures were developed to analyze neural components of cognitive or judgmental processes. Following unilateral temporal lobectomy, P300 amplitude was found to be inversely proportional to stimulus probability. With auditory stimuli, P300 activity was essentially identical for both left and right temporal patients. In patients with left temporal surgery, smaller P300s were observed, owing to a negative shift which emerged approximately 90 msec after stimulus onset.

For the visual modality, right temporal patients manifested smaller P300s than left temporal or normal subjects. There were no consistent hemispheric asymmetries which distinguished the left and right temporal patients, suggesting more than one neurogenerator of the P300 event, independent of lateral or mesial temporal structures. Processing of auditory and visual material is at least, to some extent, lateralized or hemispheric dependent.

P300 activity was also studied in normal children and patients with Turner's syndrome. Wave forms from some of the 18 and 20 year old female patients resembled the patterns of normal, however, much younger children or those entering the age of puberty. These results underscore the role of sex hormones in the development of neuropsychological processes.

The developmental course of the P300 with normal children revealed a striking change in frontal negative slow wave across the age spectrum. The amplitude and duration of this negative waveform decreased with increasing age and was inversely related to stimulus event probability. Within

conditions involving time and judgment, the P300 became more broad and peaked with advancing age. The changes in frontal negative slow wave were consistent with data from other reports, suggesting a maturation of frontal negativity which continues over the entire lifespan, and parenthetically, is altered by presenile dementia.

Visual, spatial, and constructional abilities were also examined with neuropsychiatric patients, those with Alzheimer's (AD) or Huntington's (HD) disorder. However, the pattern of deficits was different; HD patients exhibited relatively greater impairment on tests of spatial judgment (egocentric in comparison with extrapersonal spatial tasks) whereas AD patients showed the reverse pattern. These findings, viewed in the context of studies of patients with frontal vs parietal lobe lesions, implicate degeneration of frontal striatal mechanisms in HD, and the primary dysfunction in AD is associated with atrophy of cortical association regions.

The central theme of investigations with neuropsychiatric patients indicated that, at least during the early stages, patients with Alzheimer's disease may present with qualitatively different cognitive profiles, corresponding regions of neuropathology, and patterns of decline. As a result, questions concerning the status of cognition and memory in these patients can not be meaningfully or adequately addressed if they are treated as a homogeneous group.

Standardized and experimental verbal perceptuomotor tests were administered to 43 AD patients and revealed marked individual differences. A factor and a cluster analysis of the data identified several subgroups, verified by a discriminant analysis which correctly reclassified 42 of the 43 patients. Three qualitatively different groups were identified: patients with relatively equal, verbal and visuospatial impairment; patients with severe semantic memory deficits concurrent with intact visuoconstructive skills; and a third group was characterized by greater impairment of constructional skills relative to their ability to access semantic knowledge. This was associated with radiographic changes with the regional positron emission tomography data ( $^{18}\text{F}$  FDG). The data revealed symmetrical hypometabolism of the temporal and parietal cortex in globally impaired patients; relatively greater hypometabolism of the right temporal and parietal regions in patients with visuoconstructive deficits; and hypometabolism of the left temporal lobe in the semantic memory group.

**Episody Memory:** As expected, this short-term memory function was generally impaired for all groups for both verbal material (recall of stories, paired-associates, recall and recognition of word lists) and nonverbal information (reproduction and recognition of complex figures). However, a material-specific deficit limited to verbal material was found in some patients from the semantic-memory group which later progressed to a global impairment by the time retest.

**Semantic Memory:** The groups differed with respect to word-finding ability and its relation to other cognitive and episodic memory deficits. Analysis of the fluency responses and naming errors suggested that, even in the most impaired patients, access to broad categorical knowledge may be preserved. Evidence in support of this possibility was obtained by demonstrating that these patients could sort pictorial objects into appropriate categories and answer questions about superordinate features (living or man-made?) and a specific category (food, animal, or tool?). However, errors occurred when required to answer yes/no questions probing knowledge of specific attributes (e.g., it is used to cut things? [for a picture of a saw] ).

**Procedural Learning:** Patients were presented with an apparatus consisting of a 10 x 10 matrix of metal disks which, when touched by a metal stylus, signaled either a correct (low tone) or incorrect (high tone) choice. Subjects were required to discover and learn a fixed route and to remember and apply simple rules (one step at a time, no diagonal moves, return to the previous position after an error). A double dissociation was found in that patients from the semantic-memory group were able to learn the maze at a normal rate, but made a large number of rule-breaking errors (rarely committed by normals); patients with severe constructional deficits were unable to learn the route, but honored the rules.

There was a study of a single AD patient with an unusual constructional disorder and a global, episodic memory impairment, but relatively intact access to semantic memory and visual-recognition ability. While his reproduction of a complex geometric figure was severely impaired, this deficit was dissociated to a remarkable degree from his ability to draw complex and meaningful scenes. Thus, this patient's ability to tap a formerly acquired perceptual motor skill was dependent, at least in part, on the meaningfulness of the material. Therefore, even within the domain of a relatively circumscribed ability (copying visual material), both preserved and impaired functioning can be observed and related to the integrity of other cognitive systems (e.g., semantic memory).

Cerebral dysfunctioning of frontal mechanisms has been implicated in obsessive-compulsive symptomatology. Guided by this hypothesis, a series of perceptual and memory tasks were developed and administered to preadolescent and adolescent obsessive-compulsive patients (n=26) and matched normal subjects (n=24). The neuropsychiatric patients consistently did poorly with spatial learning and memory tasks, and procedures requiring left-right directional judgments or imaginary self-rotation in space. Organizational defects and atypical strategies were commonly observed with the neuropsychiatric subjects. The obsessive-compulsive patients also showed elevated thresholds for visual material tachistoscopically projected to the left, right, and central fields, and a sharp ear asymmetry with dichotic recall. These data were interpreted in the context of an inhibition-disinhibition dyscontrol. Defective neural regulators may propel automatic-stereotypic expressions of ideative and ritualistic behaviors in obsessive-compulsive disorders.

## NEURONAL EXCITABILITY SECTION

The Neuronal Excitability Section is undertaking a series of studies to determine whether the breakdown of the cytoskeletal protein fodrin is involved in the seizure focus. Emphasis will be placed on studying the structure of fodrin through antibody and electrophoresis techniques.

The question of the involvement of fodrin is addressed at several levels comparing epileptic focus to normal brain in each case:

1. Measurement of fodrin, for which gel electrophoresis and antibody techniques are used.
2. Calcium influx for which synaptoneurosome preparations in conjunction with radioactive calcium will be used.
3. Calpain the protease which breaks down fodrin will be evaluated by the  $^{14}\text{C}$ -casein breakdown technique.
4. Measurement of resting membrane potentials will be carried out in synaptoneurosomes using the  $^3\text{H}$ -tetraphenyl phosphonium technique.
5. Measurement of glutamate receptors on the synaptoneurosomes will be performed by the binding of  $^3\text{H}$ -4-amino phosphorobutyric acid.

Synaptoneurosomes were prepared from kindled and sham operated rats. The brain parts used were amygdala, substantia nigra, cortex, olfactory bulb and hippocampus. Using the  $^3\text{H}$ TPP<sup>+</sup> methodology membrane potentials were found to be in the expected range of -60 to -70mV. However under the experimental conditions used, we were unable to demonstrate any differences in membrane potentials in the kindled vs sham operated rats. The failure to demonstrate a difference may be due to several factors: 1) exposing the tissue to non physiological conditions during the preparation of the synaptosomes may have destroyed any inherent membrane potential differences; 2) the membrane potential difference is localized to a small subgroup of neurons and hence cannot be detected in a more crude preparation of whole amygdala, cortex, etc. Therefore, the experiments are being tried in several other ways which may yield increased sensitivity and better detection: a) infusion of the rat brain with  $^3\text{H}$ TPP<sup>+</sup> following the breakdown of the blood brain barrier with mannitol, followed by quantitative autoradiography, b) infusion of the brain with voltage sensitive dyes followed by visualization under a fluorescence microscope. While the TPP<sup>+</sup> assay for membrane potential was being set up this compound was tested in the presence of a number of neurotransmitters to determine whether the changes in uptake of TPP<sup>+</sup> reflected the known electrophysiology of these compounds. Depolarization was detected in the presence of arecoline, glutamate and VIP. Hyperpolarization was detected in the presence of muscimol. The one exception among the compounds tested was Substance P. This compound caused hyperpolarization of the synaptoneurosome membrane as opposed to the expected depolarization. When the rats were treated reserpine, and synaptoneurosomes prepared the

excitatory effect of Substance P could be detected. This finding indicates that a catecholaminergic could be involved in the hyperpolarization mediated by Substance P. It is also an indication that the system may be used for the study of the mechanisms of transmitter action. Several antiepileptic drugs were also tested for their effects on the membrane potential in synaptoneurosome. At concentrations of  $10^{-10}$  to  $10^{-6}$  M phenytoin hyperpolarized the membranes, whereas at  $10^{-6}$  to  $10^{-5}$  M phenytoin depolarized the membranes. This observation is similar to that reported in the literature following *in vivo* studies. Carbamazepine from  $10^{-10}$  to  $10^{-5}$  M had no effect on the membrane potential. Valproic acid gave a 20mV hyperpolarization at  $10^{-7}$  M. The TPP+ method appears to be an interesting way of investigating neurotransmitter and drug effects and further studies along these lines are underway.

A gel electrophoresis method using 7.5% polyacrylamide gel was established to determine fodrin. A doublet which stains with coomassie blue at the 240 000 MW appears at the synaptic membranes prepared with SDS. This spot corresponds to the location of fodrin published in the literature. The identity of this protein will be double checked by immunoblotting. Once this is done rat brain dissected during level 5 seizure will be checked for differences in fodrin in the intact animal versus the seizing animal. In addition, similar studies are being undertaken with fodrin conjugated to rhodamine dye where this compound then can be observed under fluorescence microscopy.

Amino acids, catecholamines and neuropeptides were determined in the focal and nonfocal areas from the temporal lobe of patients with intractable epilepsy. The amino acids determined with the Beckman automated amino acid analyzer showed no differences between the focus and nonfocus. However, differences could still exist in the turnover of these amino acids from slices.

Catecholamines showed some interesting differences. Norepinephrine, dopamine and dopa were all increased in the epileptic focus versus the nonfocus. The following values were obtained from five patients (mean  $\pm$  SD): DOPA  $24.8 \pm 5.3$  ng/g in the focus vs  $9.5 \pm 4.0$  ng/g in the nonfocus ( $p < 0.01$ ); dopamine  $14.6 \pm 11.4$  ng/g in the focus vs  $6.1 \pm 3.7$  ng/g in the nonfocus ( $p < 0.01$ ); norepinephrine  $19.7 \pm 11.3$  ng/g in the focus vs  $9.3 \pm 6.2$  ng/g in the nonfocus ( $0.05 < p < 0.1$ ). These findings agree with the findings of Sherwin et al., who showed that tyrosine hydroxylase was elevated in the epileptic focus. This is the first time that these catecholamines have been measured in the human cortex.

Among the peptides measured somatostatin was elevated, VIP and atrial natriuretic factor were decreased, and Substance P was unchanged in the focus when compared with the nonfocus. Somatostatin was  $47.1 \pm 11.4$  pg/g in the focus vs  $26.9 \pm 13.9$  pg/g in the nonfocus. VIP was  $39.4 \pm 14.8$  pg/g in the focus vs  $52.4 \pm 19.3$  pg/g in the nonfocus. Substance P was  $12.4 \pm 1.0$  pg/g in the focus vs  $15.6 \pm 5.4$  pg/g in the nonfocus.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER 201 NS 02318-09 MNB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical Pharmacology of Antiepileptic Drugs		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  P.I.: Roger J. Porter, M.D., Neurologist, Chief, MNB, IRP, NINCDS and Head, CES, MNB, IRP, NINCDS Others: William Theodore, M.D., Neurologist, CES, MNB, IRP, NINCDS Susumu Sato, M.D., Neurologist, CES, MNB, IRP, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Medical Neurology Branch, IRP, NINCDS		
SECTION Clinical Epilepsy Section (CES)		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The Clinical Epilepsy Section has begun to place more emphasis on the <u>clinical pharmacology</u> of new <u>antiepileptic drugs</u>, with somewhat diminished studies of commonly-used medical therapies. Pilot studies are now complete on felbamate (2-phenyl 1-1,3 propanedial dicarbamate). The two patients studied are continuing on a long-term follow-up protocol. Following completion of the pilot study, the randomized placebo-controlled double-blind study was initiated. The purpose of this study is to obtain definitive information regarding the efficacy of felbamate in patients with uncontrolled partial seizures. The unique three period crossover design allows unbiased estimates of drug effects even in the presence of a carry-over effect from one period to the next. A single patient has completed the study. The patient exhibited fewer seizures during the felbamate period and has continued on the medication as an outpatient. In a separate study of patients with partial seizures, a pilot effort will be undertaken to evaluate a very old compound, dapsone, for its effect on control of partial seizures. This anti-leprosy drug recently demonstrated efficacy in animal models--such as the maximal electroshock--and its protective index is promising. The study will evaluate the pharmacology of the drug in humans, identify drug interactions with current antiepileptic drugs, establish the maximum tolerated dose, and obtain preliminary assessment of efficacy of the drug. This study will be carried out in both inpatient and outpatient facilities, but with outpatient emphasis. Such will permit a long-term follow up of the possible usefulness of this drug in patients with partial seizures. Recent studies are complete in the evaluation of phenytoin pharmacokinetics and of phenytoin protein plasma binding. In the latter study, the Section demonstrated that there is little clinical rationale for measuring free rather than total levels in most patients taking the medication. A study of carbamazepine and its metabolites is continuing; an understanding of the major metabolite, carbamazepine 10-11 epoxide, is sought.           </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02236-11 MNB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diagnostic and Therapeutic Reevaluation of Patients with Intractable Epilepsy

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Co PI: Roger J. Porter, M.D., Neurologist, Chief, MNB, IRP, NINCDS

Co PI: William H. Theodore, M.D., Neurologist, Senior Investigator, CES, MNB, IRP, NINCDS

Others: Susumu Sato, M.D., Neurologist, Senior Investigator, CES, MNB, IRP, NINCDS

## COOPERATING UNITS (If any)

Office of Administrative Management; Clinical Center, NIH

Office of the Clinical Director, NINCDS

## LAB/BRANCH

Medical Neurology Branch, IRP, NINCDS

## SECTION

Clinical Epilepsy Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, 20892

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

1.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Clinical Epilepsy Section has been developing and testing new techniques to improve seizure control, medication tolerance, and rehabilitation in patients with severe epilepsy. Patients with uncontrolled seizures are admitted for a complete evaluation, including simultaneous video and telemetered EEG recording of seizures, daily determinations of antiepileptic drug serum concentrations, positron emission tomography (PET), magnetic resonance imaging (MRI), and magnetoencephalography (MEG). A specific seizure diagnosis is established allowing each patient to be assigned to an appropriate research protocol and therapy. CSF biochemistry, and neurochemistry of temporal lobe specimens resected from patients with uncontrolled seizures, are being studied.

PET in patients with localized brain lesions has demonstrated focal hypometabolic cerebral areas corresponding to the interictal seizure EEG focus. In some patients, PET has been able to detect a focus when other methods have failed. Studies of patients during partial seizures have shown a change from hypo to hypermetabolism at the site of the focus. In the Lennox-Gastaut syndrome, PET has revealed the existence of two separate metabolic patterns despite clinical seizure similarity. PET has shown that antiepileptic drugs reduce cerebral glucose utilization.

PET studies allow more definitive overall identification of the epileptic lesion and suggest new avenues of investigation into the basic mechanisms of the epilepsies. MRI may show small structural lesions underlying PET hypometabolism even when CT is normal. Further studies will elucidate the relation of metabolic and pathologic changes. MEG may have the potential to accurately localize the subsurface origin of spikes. EEG provides little information on the spatial distribution of epileptiform in cortical depths; MEG may be superior.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02562-04 MNB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) <b>Neurophysiological Bases of Phonatory Pathology</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: Christy Ludlow, Ph.D.	Speech Pathologist	SPU, HMCS, MNB, NINCDS
Others: Nadine P. Connor, M.A. Ralph F. Nauntun, M.D. Michael Baker, M.D. GERALYN Schulz, M.A.	Speech Pathologist Otolaryngologist Neurologist Speech Pathologist	SPU, HMCS, MNB, NINCDS CDP, NINCDS OCD, NINCDS SPU, HMCS, MNB, NINCDS
COOPERATING UNITS (if any)  OCD NINCDS, CDP NINCDS		
LAB/BRANCH Medical Neurology Branch		
SECTION Human Motor Control		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.00
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             The purpose is to determine the neurophysiological bases of <u>phonatory control</u> in voice disorders. The primary emphasis has been on <u>spasmodic dysphonia</u>, an idiopathic laryngeal focal dystonia. Following a previous study of respiratory control during phonation in spasmodic dysphonia, a second study examined whether <u>respiratory control</u> was also affected during whisper, a nonphonatory speech task. Both in phonation and whisper, patients were slower, uncoordinated, and had abnormal <u>respiratory movements</u> in comparison with normal. These results suggest that this is a motor control disorder often involving other areas in addition to the larynx, and is not task specific. <u>Neurophysiological studies</u> of the <u>intrinsic laryngeal muscles</u> demonstrated increased tone both at rest (during respiration) and on phonation in spasmodic dysphonia. In the normal controls, <u>diazepam</u> reduced tone in laryngeal muscles in both respiration and phonation. No effects were found in the patients. An experimental <u>temporary nerve block</u> in patients altered the muscle activation levels on the opposite side of the larynx, suggesting that muscle spindle feedback is important in the pathophysiology of this disorder. Quantitative and objective procedures have been developed for <u>evaluating treatments</u> with this disorder. Patients are highly variable over time and multiple baseline measures are required.           </p>		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02563-04 MNB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Independent Aspects of Speech Timing in Neurological Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Christy Ludlow, Ph.D. Speech Pathologist SPU, HMCS, MNB, NINCDS

Others: Celia J. Bassich, M.A. Speech Pathologist SPU, HMCS, MNB, NINCDS

Nadine P. Connor, M.A. Speech Pathologist SPU, HMCS, MNB, NINCDS

M.E. Doran-Quine, Ph.D. Guest Worker SPU, HMCS, MNB, NINCDS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Medical Neurology Branch

## SECTION

Human Motor Control

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

.95

## PROFESSIONAL:

.95

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Perceptual and acoustic studies of Parkinson's disease patients' productions of place contrasts in CV syllables were extended from isolated productions of /ba/, /da/ and /ga/ to continuous productions of /pa/, /ta/ and /ka/ to determine whether greater difficulties occur in continuous speech. Intelligibility scores of listeners on a forced choice identification task demonstrated normal intelligibility. Acoustic measures of first and second formants, rate of change and voice onset time also resulted in very few differences from normal. This indicated that these patients have few errors in stop consonants place of articulation contrasts in controlled speech testing. These laboratory results are not commensurate with clinical impressions of poor speech intelligibility of patients during conversation. Two new types of studies have been initiated this year. In one, perceptual and acoustic measures will be made of stops and fricatives in CV and VC syllables embedded in extended speech phrases. In the other, the effects of word frequency, word length and speech planning on measures of speech initiation time, movement velocity and inter-articulator coordination are being studied in normal adults and Parkinson's disease patients.

A pilot study of lip and jaw movement velocity and displacement in patients with tardive dyskinesia examined movement during voluntary speech tasks. The same maximum displacement points were reached but at lower velocities suggesting that voluntary movement is affected by this disorder.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02564-04 MNB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Relationships between Language and Speech Deficits in Neuropathologies</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: Christy Ludlow, Ph.D.	Speech Pathologist	SPU, HMCS, MNB, NINCDS
Others: Grace Yeni-Komshian, Ph.D. Andres Salazar, M.D.	Neurolinguist Neurologist	Guest Researcher, MNB VHIS, Walter Reed Army Medical Center
COOPERATING UNITS (if any)  VHIS, Walter Reed Army Medical Center, Washington, D.C.		
LAB/BRANCH Medical Neurology Branch		
SECTION Human Motor Control		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS .20	PROFESSIONAL .20	OTHER 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The purpose is to determine the <u>brain organization</u> and inter-dependence between <u>speech production</u>, <u>speech perception</u> and <u>language</u> in adults. <u>CT scan data</u> from the Vietnam Head Injury Study provide information on lesion topography following <u>penetrating missile wounds</u>. Two studies were completed this year. In persistent <u>speech dyspraxia</u>, the lesions all involved the <u>primary</u>, <u>association motor cortices</u>, and the <u>pyramidal</u> and <u>association white matter tracts</u> in the left hemisphere. Lesions in head injured controls without speech disorders were not as extensive and did not involve all of these structures in the left hemisphere.</p> <p>A study of <u>speech perception</u> employed a <u>temporal resolution</u> gap detection task between /s/ and /a/ and a <u>temporal order</u> task requiring /sa/ or /as/ identification at different inter-stimulus onset intervals. Deficits in temporal resolution were associated with lesions in either hemisphere and did not depend upon auditory area involvement. Deficits on the temporal order task occurred only when lesions involved the <u>primary auditory</u> and <u>auditory association</u> areas in the <u>left hemisphere</u>. Therefore, only temporal order perception and not temporal resolution aspects of speech perception are dependent upon the language areas of the left hemisphere.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>			PROJECT NUMBER Z01 NS 02667-02 MNB		
PERIOD COVERED October 1, 1985 through September 30, 1986					
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Physiological Analysis of Involuntary Movements					
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)					
P.I.:	Mark Hallett, M.D.	Clinical Director	OCD	ODIR	IRP
		Chief	MDU	HMCS	MNB
				IRP	NINCDS
Others:	John Ravits, M.D.	Medical Staff Fellow	OCD	ODIR	IRP
	Michael Baker, M.D.	Medical Staff Fellow	OCD	ODIR	IRP
	Jerome Sanes, Ph.D.	Senior Staff Fellow	MDU	HMCS	MNB
	Leo Cohen, M.D.	Visiting Associate	MDU	HMCS	MNB
	Robert Spitzer, M.D.	Medical Staff Fellow	MDU	HMCS	MNB
				IRP	NINCDS
COOPERATING UNITS (if any) Department of Nuclear Medicine, Clinical Center					
LAB/BRANCH Medical Neurology Branch, Intramural Research Program					
SECTION Movement Disorders Unit, Human Motor Control Section					
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892					
TOTAL MAN-YEARS: 1.8		PROFESSIONAL: 1.6		OTHER: 0.2	
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews					
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Myoclonus</u> and a number of other <u>rapid involuntary movements</u> have been difficult to classify clinically. Clinical and physiological analysis of a continuing series of patients has led to new classifications and pathophysiological insights. A series of patients with <u>epilepsia partialis continua</u> have been studied; the pathophysiology is heterogeneous, but in most electrophysiological techniques can identify an excitable region in cerebral cortex. A series of patients with <u>adult onset tic</u> have been analyzed and clinical rules have been formulated to make this diagnosis. <u>Positron emission tomography</u> (PET) studies of patients with <u>palatal myoclonus</u> have revealed that the inferior olives are hyperactive. PET studies of patients with <u>hemiballismus</u> have revealed that hypoactivity of the ipsilateral striatum occurs as a result of the lesion in the subthalamic nucleus.         </p> <p>           In studies of <u>postural action tremors</u>, we have been analyzing the amplitude of the tremor as a function of the precise posture of the limbs. For many tremors, such as <u>cerebellar postural tremor</u>, the tremor is worse when the arms are near the body and the hands are pointing towards each other. For other tremors this is not true, and the clinical and physiological significance of this is being explored.         </p> <p>           Task specific focal <u>dystonias</u> of the hands such as <u>writer's cramp</u> and <u>pianist's cramp</u> have been analyzed and a number of physiological characteristics have been defined. There appears to be diminished ability to control the fingers independently and gating of somatosensory evoked potentials with voluntary movement is abnormal. The spasms themselves have been characterized into different patterns. Abnormalities of the <u>blink reflex</u> have been identified in dystonic disorders. We have verified this in a number of our own patients and are now applying this test to the patients with focal hand cramps.         </p>					

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 NS 02668-02 MNB
<b>PERIOD COVERED</b> October 1, 1985 through September 30, 1986		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) Trial of Isoniazid for Action Tremor		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Mark Hallett, M.D. Clinical Director	OCD ODIR IRP NINCDS Chief MDU HMCS MNB IRP NINCDS
Others:	John Ravits, M.D. Medical Staff Fellow	OCD ODIR IRP NINCDS
	Michael Baker, M.D. Medical Staff Fellow	OCD ODIR IRP NINCDS
	Jerome Sanes, Ph.D. Senior Staff Fellow	MDU HMCS MNB IRP NINCDS
	Leo Cohen, M.D. Visiting Associate	MDU HMCS MNB IRP NINCDS
	Robert Spitzer, M.D. Medical Staff Fellow	MDU HMCS MNB IRP NINCDS
<b>COOPERATING UNITS</b> (if any) None		
<b>LAB/BRANCH</b> Medical Neurology Branch, Intramural Research Program		
<b>SECTION</b> Movement Disorders Unit, Human Motor Control Section		
<b>INSTITUTE AND LOCATION</b> NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	0.3	PROFESSIONAL: 0.2 OTHER: 0.1
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)  Previous studies have shown utility of <u>isoniazid</u> for ameliorating <u>cerebellar postural tremor</u> in patients with <u>multiple sclerosis</u> . Current studies are aimed at identifying whether patients with <u>action tremors</u> of other types are also benefitted. A double-blind placebo-controlled, cross-over trial is in progress. Thirteen patients have now been studied. Isoniazid does appear to help some of these cases, but we do not have enough experience yet to predict the response.		

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02669-02 MNB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Analysis of Voluntary Movement

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.	Clinical Director		ODC	ODIR	IRP	NINCDS
		Chief	MDU	HMCS	MNB	IRP	NINCDS
Others:	John Ravits, M.D.	Medical Staff Fellow		ODC	ODIR	IRP	NINCDS
	Michael Baker, M.D.	Medical Staff Fellow		ODC	ODIR	IRP	NINCDS
	Jerome Sanes, Ph.D.	Senior Staff Fellow	MDU	HMCS	MNB	IRP	NINCDS
	Leo Cohen, M.D.	Visiting Associate	MDU	HMCS	MNB	IRP	NINCDS
	Robert Spitzer, M.D.	Medical Staff Fellow	MDU	HMCS	MNB	IRP	NINCDS

## COOPERATING UNITS (if any)

Department of Rehabilitation Medicine, Clinical Center  
 Department of Nuclear Medicine, Clinical Center

## LAB/BRANCH

Medical Neurology Branch, Intramural Research Program

## SECTION

Movement Disorders Unit, Human Motor Control Section

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

1.8

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies in the Gait Laboratory of the Department of Rehabilitation Medicine have focussed on the control of balance. Simultaneous measurement of body angles, foot-floor forces and multiple EMGs are possible. Studies in normal subjects have revealed insights into the biomechanical effects of postural muscle activity. Similar studies have been initiated in patients with cerebellar disturbance and Parkinson's Disease. Two patients with orthostatic tremor have also been investigated.

In patients with Parkinson's Disease there was a positive correlation between circulating levels of dopamine and cognitive motor capabilities in a choice reaction time situation but not in a simple reaction time situation. In normal subjects, triggered muscle responses produced by stopping voluntary movements were similar to muscle responses generated when subjects initiated a voluntary movement from the stopped location, thereby suggesting that triggered and voluntary reactions are mediated by similar central nervous system mechanisms.

The study of motor control in hemiplegia is being planned and apparatus is being prepared. Patients with discrete brain lesions will be studied; patients with strokes will be the main group, and many patients will be followed serially from onset of the disorder to recovery. Preliminary studies with positron emission tomography (PET) of normal subjects have shown that it is difficult to identify cortical areas active with voluntary movement using fluoro-deoxy-glucose (FDG) as the tracer. Further studies are planned with oxygen-15-water which will probe blood flow rather than glucose metabolism.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 NS 02711-01 MNB																												
<b>PERIOD COVERED</b> October 1, 1985 through September 30, 1986																														
<b>TITLE OF PROJECT</b> ( <i>#80 characters or less. Title must fit on one line between the borders.</i> ) Utility and Physiology of Botulinum Toxin for Involuntary Movement Disorders																														
<b>PRINCIPAL INVESTIGATOR</b> ( <i>List other professional personnel below the Principal Investigator. (Name, title, laboratory, and institute affiliation)</i> )																														
P.I.: Mark Hallett, M.D.  Others: John Ravits, M.D. Michael Baker, M.D. Leo Cohen, M.D. Christy Ludlow, Ph.D. Ralph Naunton, M.D.	Clinical Director Chief Medical Staff Fellow Medical Staff Fellow Visiting Associate Speech Pathologist Otolaryngologist	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: center;">OCD</td> <td style="text-align: center;">ODIR</td> <td style="text-align: center;">IRP</td> <td style="text-align: center;">NINCDS</td> </tr> <tr> <td style="text-align: center;">MDU</td> <td style="text-align: center;">HMCS</td> <td style="text-align: center;">MNB</td> <td style="text-align: center;">IRP</td> </tr> <tr> <td style="text-align: center;">OCD</td> <td style="text-align: center;">ODIR</td> <td style="text-align: center;">IRP</td> <td style="text-align: center;">NINCDS</td> </tr> <tr> <td style="text-align: center;">OCD</td> <td style="text-align: center;">ODIR</td> <td style="text-align: center;">IRP</td> <td style="text-align: center;">NINCDS</td> </tr> <tr> <td style="text-align: center;">MDU</td> <td style="text-align: center;">HMCS</td> <td style="text-align: center;">MNB</td> <td style="text-align: center;">IRP</td> </tr> <tr> <td style="text-align: center;">SPU</td> <td style="text-align: center;">HMCS</td> <td style="text-align: center;">MNB</td> <td style="text-align: center;">IRP</td> </tr> <tr> <td style="text-align: center;"></td> <td style="text-align: center;">CDP</td> <td style="text-align: center;">EAP</td> <td style="text-align: center;">NINCDS</td> </tr> </table>	OCD	ODIR	IRP	NINCDS	MDU	HMCS	MNB	IRP	OCD	ODIR	IRP	NINCDS	OCD	ODIR	IRP	NINCDS	MDU	HMCS	MNB	IRP	SPU	HMCS	MNB	IRP		CDP	EAP	NINCDS
OCD	ODIR	IRP	NINCDS																											
MDU	HMCS	MNB	IRP																											
OCD	ODIR	IRP	NINCDS																											
OCD	ODIR	IRP	NINCDS																											
MDU	HMCS	MNB	IRP																											
SPU	HMCS	MNB	IRP																											
	CDP	EAP	NINCDS																											
<b>COOPERATING UNITS</b> ( <i>if any</i> ) None																														
<b>LAB/BRANCH</b> Medical Neurology Branch, Intramural Research Program																														
<b>SECTION</b> Movement Disorders Unit, Human Motor Control Section																														
<b>INSTITUTE AND LOCATION</b> NINCDS, NIH, Bethesda, Maryland 20892																														
<b>TOTAL MAN-YEARS:</b> 0.5	<b>PROFESSIONAL:</b> 0.4	<b>OTHER:</b> 0.1																												
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																														
<b>SUMMARY OF WORK</b> ( <i>Use standard unreduced type. Do not exceed the space provided.</i> )  Botulinum toxin injected in small doses directly into muscle binds to the <u>neuromuscular junction</u> and inactivates it for approximately 3 months. This treatment has been demonstrated to be useful for <u>strabismus</u> and <u>blepharospasm</u> , but there has not been a complete understanding of its mechanism of action.  Studies of utility of botulinum toxin have been carried out in <u>spasmodic dysphonia</u> and <u>writer's cramp</u> (and its variants such as pianist's cramp). Treatment appears efficacious for both. Further studies are planned with <u>adult stuttering</u> .  Studies of physiology of the mode of action have been carried out in spasmodic dysphonia, writer's cramp, blepharospasm and <u>hemifacial spasm</u> . These studies are currently incomplete.																														



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>			<b>PROJECT NUMBER</b> Z01 NS 02712-01 MNB			
<b>PERIOD COVERED</b> October 1, 1985 through September 30, 1986						
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) Non-invasive Stimulation of Human Central Nervous System						
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)						
P.I.:	Mark Hallett, M.D.	Clinical Director	OCD	ODIR	IRP	NINCDS
		Chief	MDU	HMCS	MNB	IRP
Others:	Jerome Sanes, Ph.D.	Senior Staff Fellow	MDU	HMCS	MNB	IRP
	Leo Cohen, M.D.	Visiting Associate	MDU	HMCS	MNB	IRP
						NINCDS
<b>COOPERATING UNITS</b> (if any) None						
<b>LAB/BRANCH</b> Medical Neurology Branch, Intramural Research Program						
<b>SECTION</b> Movement Disorders Unit, Human Motor Control Section						
<b>INSTITUTE AND LOCATION</b> NINCDS, NIH, Bethesda, Maryland 20892						
<b>TOTAL MAN-YEARS:</b> 0.2		<b>PROFESSIONAL:</b> 0.1		<b>OTHER:</b> 0.1		
<b>CHECK APPROPRIATE BOX(ES)</b>						
<input checked="" type="checkbox"/> (a) Human subjects		<input type="checkbox"/> (b) Human tissues		<input type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors						
<input type="checkbox"/> (a2) Interviews						
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.) <p>Recently techniques have become available for the <u>non-invasive stimulation</u> of the <u>human cortex</u> and <u>human spinal cord</u>. A device has been manufactured which produces a high voltage, extremely brief pulse which can penetrate skull and activate central nervous system without excessive pain. Previous studies have shown it useful in some circumstances where evaluation of the speed and integrity of <u>motor pathways</u> is valuable.</p> <p>We have just begun to use this technique. Our first project is to see if we can map the <u>motor cortex</u>.</p>						

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02630-03 MNB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Clinical, Genetic, and Biochemical Studies of Familial Alzheimer Disease</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: R.J. Polinsky, M.D. Chief CNS, MNB, NINCDS		
Others: L.E. Nee, M.S.W. Social Science Analyst OCD, NINCDS R.T. Brown, M.D. Medical Staff Fellow CNS, MNB, NINCDS J.H. Robbins, M.D. Senior Investigator D, DCBD, NCI E.S. Gershon, M.D. Chief CNG, NIMH G. Di Chiro, M.D. Chief NIS, OD, IRP, NINCDS J. Grafman, Ph.D. Psychologist CLPS, MNB, NINCDS		
COOPERATING UNITS (if any) Lab. of Histopath., La Salpetriere (J. Foncin); Genetics Unit, Dept. of Neurology, Mass. Gen. Hosp. (J. Gusella, P. Hyslop); Dept. of Genetics, Indiana Univ. (M. Conneally); Neuropath. Lab., Johns Hopkins Hosp. (D. Price, R. Struble), Dept. of Neurology, Univ. of Vermont (S. Robison, W. Bradley); CON'T		
LAB/BRANCH Medical Neurology Branch, IRP, NINCDS		
SECTION Clinical Neuropharmacology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 4.0		PROFESSIONAL: 3.0
		OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Alzheimer's disease</u> (AD) is the most common cause of irreversible, chronic <u>dementia</u> . Although AD may be familial in only one third of all cases, the main justification for studying autosomal dominant cases lies in the accuracy of diagnosis which may be inferred through post-mortem examination of other affected family members. Neuropathological examination in our cases of dominantly inherited AD reveals changes in Sommer's sector of the hippocampus in half of the examined cases. There is marked variability among 4 cases within one large kindred. No definite evidence for AD was found in the brain of an asymptomatic at-risk family member. Previous genetic studies have not clarified the role of inheritance. <u>Skin fibroblast</u> and <u>peripheral blood lymphoblast</u> cultures are being established from members of large kindreds with familial AD. These cultures serve as a renewable source of DNA and cell lines which can be used for genetic linkage, viability, and biochemical studies. A study of 22 <u>twin</u> pairs revealed concordance of only 7 of 17 monozygotic and also 2 of 5 dizygotic pairs suggesting that factors other than heredity are involved in AD. The development of recombinant DNA technology is being applied to genetic linkage analysis in our large kindreds with dominant AD. Approximately 70% of chromosome 21 can confidently be excluded. The Gm locus on chromosome 14 has also been excluded through the use of the D14S1 probe. Linkage analyses of other single candidate genes are in progress. Preliminary studies show that DNA repair in lymphoblasts from inherited AD patients is significantly less than in control lines. A longitudinal investigation of clinical, biochemical, metabolic, and neuropharmacological changes in affected and at-risk members of these families is being conducted. Global and regional glucose metabolic rate (CGMR), measured by <u>PET scanning</u> , were reduced in two patients with dominantly inherited AD. Supramarginal gyri and temporal lobes were involved out of proportion to other brain regions. Further studies are necessary to determine whether changes in CGMR precede clinical expression of the disease.		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 NS 02115-13 MNB
PERIOD COVERED October 1, 1985 to September 30, 1986	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Biochemical Indices of Adrenergic Function in Humans</b>	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: R.J. Polinsky, M.D. Chief CNS, MNB, NINCDS	
Others: R.T. Brown, M.D. Medical Staff Fellow CNS, MNB, NINCDS L.E. Nee, M.S.W. Social Science Analyst OCD, NINCDS I.J. Kopin, M.D. Chief IPS, NIB, NINCDS M. Hallett, M.D. Chief HMCS, MNB, NINCDS B. Pastakia, M.D. Staff Fellow DR, CC G. Di Chiro, M.D. Chief NIS, OD, IRP, NINCDS	
COOPERATING UNITS (if any) Division of Endocrinology, VA Hospital, Washington, DC (L. Recant); Neuropathology Lab, Johns Hopkins Hosp., Baltimore, MD (D. Price, J. Troncosa)	
LAB/BRANCH Medical Neurology Branch	
SECTION Clinical Neuropharmacology Section	
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20892	
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0
OTHER: 1.0	
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Autonomic nervous system</u> activity is essential for maintaining circulatory and metabolic homeostasis. In order to study <u>sympathetic nervous system</u> function and its relationship to other <u>neuroendocrine</u> systems, it is necessary to measure <u>neurotransmitter</u> , <u>hormonal</u> , and <u>peptide</u> levels in response to various stimuli. The levels of <u>norepinephrine</u> , <u>epinephrine</u> , and <u>dopamine</u> and their metabolites in various body fluids reflect the activity of the neurones from which these neurotransmitters are released. Measurement of urinary <u>catecholamine metabolites</u> and their stereospecific labelling pattern following administration of radiolabelled isomers of norepinephrine provides a means for investigating the origin of norepinephrine metabolites. <u>Cerebrospinal fluid</u> levels of monoamine metabolites can be used to assess central nervous system neurotransmitter metabolism. It is necessary to consider the origin of these metabolites to make appropriate corrections for valid interpretations of the data. These strategies have been used to study patients with <u>neurogenic orthostatic hypotension</u> and in other clinical situations in which adrenergic function is abnormal. Insulin may play a role in causing post-prandial hypotension in patients with autonomic failure. The mechanism of insulin-induced hypotension may involve release of beta-endorphin without a concomitant elevation of norepinephrine or a direct effect of insulin on brain receptors. Glucose prevents the blood pressure drop following insulin administration in patients with autonomic failure attended by central neurological signs but not in patients with isolated autonomic failure. In normal subjects most vanillylmandelic acid is derived from 3-methoxy-4-hydroxyphenylglycol. Investigation of the effects of <u>aging</u> on autonomic nervous system function is in progress. A more thorough understanding of neurotransmitter metabolism in these clinical situations leads to more rational approaches to therapy.	

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <div style="text-align: right;">Z01 NS 01658-19 MNB</div>																								
PERIOD COVERED October 1, 1985 through September 30, 1986																										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <i>Hemispheric Development and Specialization of the Intellectual Functions</i>																										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)																										
<table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: P. Fedio, Ph.D.</td> <td style="width: 20%;">Psychologist</td> <td style="width: 10%;">MN</td> <td style="width: 30%;">NINCDS</td> </tr> <tr> <td>Others: C. Cox, M.S.</td> <td>Psychologist</td> <td>MN</td> <td>NINCDS</td> </tr> <tr> <td>J. Grafman, Ph.D.</td> <td>Psychologist</td> <td>MN</td> <td>NINCDS</td> </tr> <tr> <td>C. Kufra, M.D.</td> <td>Medical Officer</td> <td>SN</td> <td>NINCDS</td> </tr> <tr> <td>A. Martin, Ph.D.</td> <td>Psychologist</td> <td>MN</td> <td>NINCDS</td> </tr> <tr> <td>P. Brouwers, Ph.D.</td> <td>Psychologist</td> <td>LPP</td> <td>NIMH</td> </tr> </table>			PI: P. Fedio, Ph.D.	Psychologist	MN	NINCDS	Others: C. Cox, M.S.	Psychologist	MN	NINCDS	J. Grafman, Ph.D.	Psychologist	MN	NINCDS	C. Kufra, M.D.	Medical Officer	SN	NINCDS	A. Martin, Ph.D.	Psychologist	MN	NINCDS	P. Brouwers, Ph.D.	Psychologist	LPP	NIMH
PI: P. Fedio, Ph.D.	Psychologist	MN	NINCDS																							
Others: C. Cox, M.S.	Psychologist	MN	NINCDS																							
J. Grafman, Ph.D.	Psychologist	MN	NINCDS																							
C. Kufra, M.D.	Medical Officer	SN	NINCDS																							
A. Martin, Ph.D.	Psychologist	MN	NINCDS																							
P. Brouwers, Ph.D.	Psychologist	LPP	NIMH																							
COOPERATING UNITS (if any) <i>Surgical Neurology Branch, IRP, NINCDS</i> <i>Laboratory of Psychology and Psychopathology, IRP, NIMH</i>																										
LAB/BRANCH <i>Medical Neurology, IRP, NINCDS</i>																										
SECTION <i>Clinical Neuropsychology</i>																										
INSTITUTE AND LOCATION <i>NINCDS, NTH, Bethesda, MD 20892</i>																										
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MAN-YEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 34%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">2.0</td> <td style="text-align: center;">1.5</td> <td style="text-align: center;">0.5</td> </tr> </table>			TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:	2.0	1.5	0.5																		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:																								
2.0	1.5	0.5																								
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"><input checked="" type="checkbox"/> (a) Human subjects</td> <td style="width: 33%;"><input type="checkbox"/> (b) Human tissues</td> <td style="width: 34%;"><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input checked="" type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input checked="" type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews																	
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<input checked="" type="checkbox"/> (a1) Minors																										
<input type="checkbox"/> (a2) Interviews																										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p style="margin: 0;">The disabling effects of chronic <u>cerebral insult</u> and <u>neuropsychiatric disorders</u> were evaluated by a broad range of <u>neuropsychological tests</u> evaluating <u>brain-behavior</u> relations in man.</p> <p style="margin: 0;">Asymptomatic long-term survivors of <u>acute lymphoblastic leukemia</u> (ALL) who received CNS preventive therapy (cranial irradiation and intrathecal chemotherapy) were studied. Based on <u>CT scan</u> findings, the patients were divided into three groups: normal scans, cortical atrophy; intracerebral calcifications. <u>Memory and learning</u> were significantly impaired in children with abnormal scans, more so for the patients with calcification. In addition, all patients with abnormal CT scans showed significant attentional dysfunctions.</p> <p style="margin: 0;">Adolescents with <u>obsessive compulsive</u> features exhibited a cluster of neuropsychological deficits which correlated with ventricular enlargement. Deficits were identified in spatial judgement and spatial learning. It was suggested that an imbalance in the inhibitory functions of the frontal lobe and limbic systems may contribute to obsessive compulsive behavior.</p> <p style="margin: 0;">Patients with <u>Tourette's Syndrome</u> were evaluated in an effort to characterize the neuropsychological defects in frontal inhibitory mechanisms. Positron emission tomographic (PET) data of regional cerebral activity from these patients will be correlated with test performance.</p>																										

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01424-20 MNB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Modulation by the Limbic System in Man

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Fedio, Ph.D.	Psychologist	MN	NINCDS
Others:	P. Brouwers, Ph.D.	Psychologist	LPP	NIMH
	C. Cox, M.S.	Psychologist	MN	NINCDS
	F. Lalonde, M.A.	Psychologist	MN	NINCDS
	E. Mohr, Ph.D.	Psychologist	MN	NINCDS
	E. Witt, Ph.D.	Psychologist	MN	NINCDS
	C. Kufka, M. D.	Medical Officer	SN	NINCDS
	A. Martin, Ph.D.	Psychologist	MN	NINCDS

## COOPERATING UNITS (if any)

Surgical Neurology Branch, IRP, NINCDS  
 Laboratory of Psychology and Psychopathology, IRP, NIMH

## LAB/BRANCH

Medical Neurology, IRP, NINCDS

## SECTION

Clinical Neuropsychology

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Emotional and cognitive characteristics were studied in epileptic patients before and following unilateral left or right temporal lobe resection. The integrity of attentional and perceptual (visual, auditory, and tactile) systems were evaluated using standard and experimental procedures. Physiological events (skin conductance) were also monitored during test performance. The research examined the role of the temporal lobe in establishing specific limbic associations between left and right hemispheres in regulating cognitive functions and emotional experiences in man.

Tachistoscopic studies identified a critical perceptual role for right temporal mechanisms, especially during initial visual processing. The left and right temporal lobes contribute differentially to specifying the identity of a stimulus and its position or orientation in space. Left temporal mechanisms encode verbal information during initial learning. Modest compensation for memory defects following temporal lobectomy may be achieved with strategies which combine overt and covert imagery with praxic encoding.

In affective sectors, left temporal patients tend to neutralize reactions to nuances with emotional coloration; right temporal patients, in contrast, rate these materials similar to normal individuals. Unlike normal individuals, however, the left and right temporal lobectomy patients were hyporesponsive to affective material as indexed by skin conductance measures. Moreover, both lobectomy groups failed to take advantage of the emotional characteristics of information to facilitate memory. These data suggest that unilateral temporal lobectomy disrupts the normal linkage of cognitive-affective associations mediated by temporal limbic interaction. There were, however, beneficial effects to surgical treatment in that patients, following temporal lobe surgery, were less deviant from normal subjects in emotional behavior.

33 MNB/IRP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

701 NS 01245-21 MNB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. Johnson, Jr., Ph.D. Psychologist MN NINCDS  
P. Fedio, Ph.D. Psychologist MN NINCDS  
Others: A. Martin, Ph.D. Psychologist MN NINCDS  
C. Kufita, M.D. Medical Officer SN NINCDS  
R. Porter, M.D. Medical Officer MN NINCDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, IRP, NINCDS  
Laboratory of Psychology and Psychopathology, IRP, NIMH

LAB/BRANCH

Medical Neurology, IRP, NINCDS

SECTION

Clinical Neuropsychology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Information processing was monitored by averaged evoked response techniques. The electrographic activity was recorded from left and right brain regions during memory and perception in normal subjects, patients with unilateral temporal lobectomy, and patients with neuropsychiatric disorders. Electroencephalographic disturbances in brain-behavior relations in psychiatric patients were also evaluated, relating left and right brain dysfunctioning to activity to maladaptive ideative and emotional reactions, respectively.

In temporal lobectomy patients, P300 amplitude was found to be inversely proportional to stimulus probability in the same way as for normal controls, and larger P300s were elicited in reaction time. For visual material, right-temporal patients manifested smaller P300s at frontal sites than either left-temporal or normal individuals. Moreover, there were no consistent hemispheric asymmetries which distinguished the left-or right-temporal patients, or either group from normal subjects. These data discount the hypothesis that medial temporal structures, including the hippocampus, serve as a sole generator of P300. More specifically the data indicate that processing of auditory and visual stimuli is dependent to a great extent on the character of the material and the integrity of left and right brain mechanisms.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 00200-32 MNB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cognitive and Emotional Profile of Neuropsychiatric Disorders

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. Fedio, Ph.D. Psychologist MNB NINCDS  
 E. Mohr, Ph.D. Psychologist MNB NINCDS  
 Others: P. Brouwers, Ph.D. Psychologist LPP NIMH  
 C. Cox, M.S. Psychologist MNB NINCDS  
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 A. Martin, Ph.D. Psychologist MNB NINCDS  
 E. Witt, Ph.D. Psychologist MNB NINCDS  
 T. Chase, M.D. Neurologist ET NINCDS

## COOPERATING UNITS (if any)

Experimental Therapeutics Branch, IRP, NINCDS  
 Laboratory of Psychology and Psychopathology, IRP, NIMH

## LAB/BRANCH

Medical Neurology, IRP, NINCDS

## SECTION

Clinical Neuropsychology

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

3.0

## PROFESSIONAL:

2.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A neuropsychological profile of dementia was drafted for individuals with Alzheimer's Disease, Huntington's Disease and 'at risk' for Huntington's Disease. The evaluations extended into memory, learning and perceptual areas, utilizing standard and experimental tasks, also establishing normative references for functional changes accompanying the aging processes.

Although Alzheimer's Disease is accompanied by marked deficits in selective attention, memory and learning, there were no qualitative differences between demented and age-matched subjects. The impairment also extended to object-naming and fluency, and AD patients performed poorly in perceiving meaning, except when the stimuli required emotional judgment. The data indicate that Alzheimer's patients may be unable to encode material; this is in sharp contrast with other amnesic disorders where the primary difficulty involves an inability to store and/or retrieve information.

Alzheimer's and Huntington's patients showed pronounced but dissimilar deficits with visuospatial and constructional tasks. The behavioral data extend neuropathologic impressions of degeneration of the frontal striatal system in Huntington's Disease, and temporo-parietal, cortical involvement in Alzheimer's Disease.

The neuropsychological test profile of Alzheimer's patients yielded different clinical subgroups or populations. Memory and learning deficits, per se, were poor indicators of group membership. One group was characterized by severely impaired verbal abilities, but with intact perceptual and constructional skills. The second group was more impaired on perceptuomotor than verbal tasks. The third group showed comparable deficiencies in both linguistic and visual spatial sectors. Positron emission tomographic and EEG data confirmed corresponding changes in left, right or bilateral regions in the posterior cerebral quadrant, respectively.

35 MNB/IRP

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <b>Z01 NS 02678-02 MNB</b>
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Involvement of Calcium, Fodrin, and Glutamate in Seizures</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <b>P.I.: S. Nadi, Ph.D., Senior Staff Fellow, NES, MNB, NINCDS</b> <b>Other: D.S. Goldstein, M.D., NHLBI, NIH</b>		
COOPERATING UNITS (if any) <b>Univ. Tennessee, Memphis, TN (A. Wyler, M.D.); Yale Univ, New Haven CT (D. Morrow, M.D.); NNMCM, Bethesda, MD (J. Morales, M.D.); Hebrew Univ, Jerusalem (D. Lichstein, Ph.D.)</b>		
LAB/BRANCH <b>Medical Neurology Branch, IRP, NINCDS</b>		
SECTION <b>Neuronal Excitability Section</b>		
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, Maryland 20892</b>		
TOTAL MAN-YEARS: <b>0.2</b>	PROFESSIONAL: <b>0.05</b>	OTHER: <b>0.15</b>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided )  <p>The work will involve the study of epileptic foci and the evaluation of the status of glutamate receptors, the cytoskeletal protein fodrin and the flux of calcium ions. The epileptic seizure has been demonstrated to occur as a result of a shift of external calcium ions to the interior of the cell. Since calcium ions are also known to activate calpain, which degrades fodrin, and is responsible for maintaining the integrity of the membrane. The question of interest in this study is to determine whether fodrin breakdown is directly linked to the spread of epileptic activity.</p> <p>In addition, the epileptic focus obtained from patients with intractable seizures will be screened for abnormalities in amino acid, catecholamine and neuropeptide levels.</p>		



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02679-02 MNB
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Plasma & CSF Levels of Neurotransmitters in Epileptics & to EKG Correlation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: S. Nadi, Ph.D., Senior Staff Fellow, NES, MNB, NINCDS Others: R. Porter, M.D., Chief, MNB, NINCDS B. Ito, Medical Staff Fellow, OCD, NINCDS		
COOPERATING UNITS (if any)		
LAB/BRANCH Medical Neurology Branch, IRP, NINCDS		
SECTION Neuronal Excitability Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.05	OTHER: 0.15
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  Blood and CSF samples will be obtained from patients with epilepsy before and after a seizure. Neurotransmitters such as catecholamines, amino acids, and peptides will be measured by HPLC and RIA techniques. These findings will be correlated to any EEG or EKG changes which occur during seizures in order that some insight into the sudden death syndrome in epilepsy might be obtained.		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02713 01 MNB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Involvement of Abnormalities in Glutamate Decarboxylase Gene in the Kindled Rat</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.I.: S. Nadi, Ph.D., Senior Staff Fellow, NES, MNB, NINCDS Others: E. Freese, M.D., Chief, Laboratory of Molecular Biology, NINCDS Dr. J. Thomas, Staff Fellow, Laboratory of Molecular Biology, NINCDS Dr. C. Banner, Staff Fellow, Laboratory of Molecular Biology, NINCDS R. Henneberry, M.D., Chief, Section of Molecular Neurobiology, NINCDS Dr. L. Vitkovic, Senior Staff Fellow, Laboratory of Molecular Biology, NINCDS		
COOPERATING UNITS (if any) Univ. Tennessee, Memphis, TN (A. Wyler, M.D.); Surgical Neurology Branch, NINCDS (C. Kufta, M.D.); Dept. Biology, UCLA, Los Angeles, CA (A. Tobin)		
LAB/BRANCH Medical Neurology Branch, IRP, NINCDS		
SECTION Neuronal Excitability Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.8	PROFESSIONAL: 0.8	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  Abnormalities in various steps of the aminobutyric acid have been reported to occur in epilepsy. The aim of the present study is to study the glutamate decarboxylase (GAD) gene by <u>in situ</u> hybridization in kindled and sham operated rats. It is anticipated that this approach will show if there are any mRNA changes due to epileptiform activity. This is a study currently being undertaken; no results are available yet.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 NS 02269-10 MN3

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Visual Evoked Potentials in Clinical Neurology and Neuro-Ophthalmology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Susumu Sato, M.D.	Medical Officer	MNB, NINCDS
OTHERS:	Douglas F. Rose, M.D.	Medical Staff Fellow	OCD, NINCDS
	Vita Alexander, REEGT	Chief, Technologist	OCD, NINCDS
	William Thomas	EEG Technologist	OCD, NINCDS

COOPERATING UNITS (If any)

Office of Clinical Director, IRP, NINCDS

LAB/BRANCH

Medical Neurology Branch, IRP, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Visual evoked potentials to checkerboard pattern, were studied in normal volunteers and patients with various neurological disorders, particularly multiple sclerosis and seizures.

A. Multiple Sclerosis: The prolongation of the major positive peak has been consistently found in patients with history of optic neuritis and in some patients even without such a history. In some patients with the history of optic neuritis who have been visually asymptomatic for many years, however, persistent prolongation or normalization of the latency has been noted.

B. Epileptic Seizures: Visual evoked potentials to be a half-visual field stimulation (studying the retrodiastmatic visual pathway) have been studied in patients with complex partial seizures. The primary goal is to predict the side of the epileptic lesion by the visual evoked potentials. The preliminary analysis showed no clear-cut predictability.

The significance of the visual evoked potentials lies in the fact that they are totally noninvasive, are useful in detecting the occult lesions and in evaluating the visual system in the context of the cortical nervous system integrity.

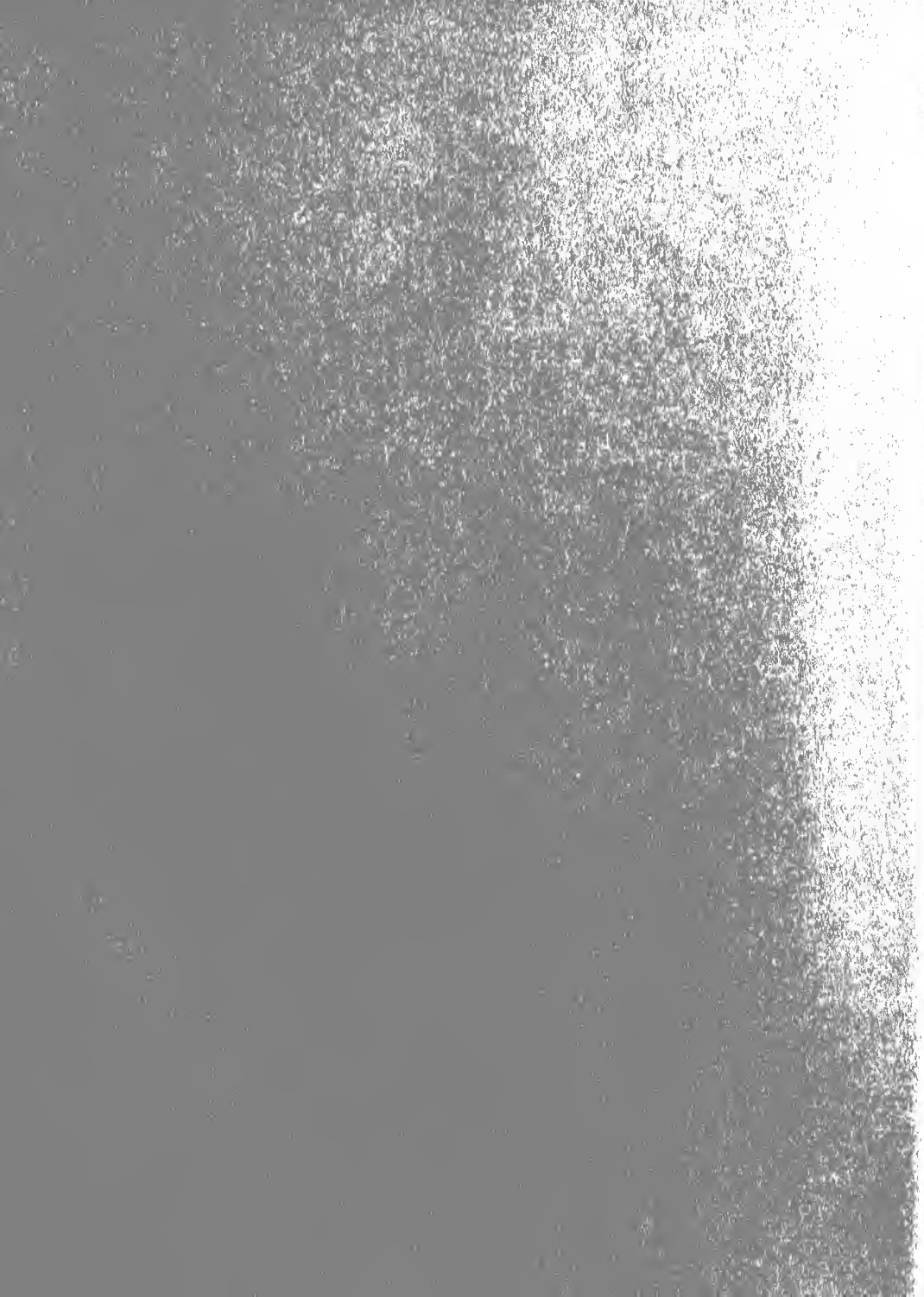
This project has been completed.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 NS 02431-07 MNB
<b>PERIOD COVERED</b> October 1, 1985 through September 30, 1986		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) Experimental Epilepsy: Seizures Produced by Kindling in Rat		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Shun-ichi Yamaguchi, Ph.D.	Psychologist	MNB, NINCDS
OTHERS: Susumu Sato, M.D. Stuart Walbridge	Medical Officer Lab Specialist	MNB, NINCDS MNB, NINCDS
<b>COOPERATING UNITS</b> (if any) Office of the Clinical Director, IRP, NINCDS		
<b>LAB/BRANCH</b> Medical Neurology Branch, IRP, NINCDS		
<b>SECTION</b> Neuronal Excitability Section		
<b>INSTITUTE AND LOCATION</b> NINCDS, NIH, Bethesda, MD 20892		
<b>TOTAL MAN-YEARS:</b> 0.8	<b>PROFESSIONAL:</b> 0.6	<b>OTHER:</b> 0.2
<b>CHECK APPROPRIATE BOX(ES)</b> <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)  <p>In rats, seizures produced by chronic stimulation (kindling) of amygdaloid complex or other nuclei were studied.</p> <p>A. Amygdaloid and globus pallidus kindling lead to notably different clinical seizure patterns in rats. Local cerebral blood flow determination by [C-14] antipyrine autoradiography indicated that globus pallidus kindling led to LCDF increases in mostly non-limbic structures, whereas amygdaloid kindling led to increases in mostly limbic structures.</p> <p>B. Different parts of rat hippocampus may not be homogenous in their functional properties. Kindling by ventral hippocampal stimulation proceeds at a much greater rate than by dorsal hippocampal stimulations. Also, lesions in ventral hippocampus appear to facilitate initial kindling process by amygdala stimulations.</p> <p>C. Certain monoclonal raised antibody to a rat hippocampal protein inhibits long-term potentiation (LTP) effect. Since LTP is indicated to be involved in kindling, effects of injecting this antibody with lateral ventricle on kindling were studied. Preliminary indications are not contradictory to this expectation, but definitive trend was not yet established.</p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 NS 02432-07 MNB
<b>PERIOD COVERED</b> October 1, 1985 through September 30, 1986		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) Brainstem Auditory Evoked potentials in Clinical Neurology		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Susumu Sato, M.D.	Medical Officer MNB, NINCDS
OTHERS:	Douglas F. Rose, M.D. Vita Alexander, REEGT	Medical Staff Fellow Chief, Technologist OCD, NINCDS OCD, NINCDS
<b>COOPERATING UNITS</b> (if any) Office of the Clinical Director, IRP, NINCDS		
<b>LAB/BRANCH</b> Medical Neurology Branch, IRP, NINCDS		
<b>SECTION</b> Neuronal Excitability Section		
<b>INSTITUTE AND LOCATION</b> NINCDS, NIH, Bethesda, MD 20892		
<b>TOTAL MAN-YEARS:</b> 0.5	<b>PROFESSIONAL:</b> 0.2	<b>OTHER:</b> 0.3
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.)  Brainstem auditory evoked responses to clicks were studied in normal volunteers and in patients with various neurological disorders.  A. <u>Multiple Sclerosis</u> : In some patients with multiple sclerosis, prolongation of latencies, distortion of waveforms or disappearance of some components were noted.  B. <u>Epilepsy</u> : In patients with complex partial seizures no significant changes in terms of latencies or waveform have been noted.  The significance of this project is that it can provide information on the functional integrity of the brainstem.  This project has been completed.		









# ANNUAL REPORT

October 1, 1985 through September 30, 1986

## Neuroepidemiology Branch

National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report  
October 1, 1985 through September 30, 1986  
Neuroepidemiology Branch  
Intramural Research Program  
National Institute of Neurological and Communicative  
Disorders and Stroke

Bruce S. Schoenberg, M.D., Dr.P.H., Chief

The Neuroepidemiology Branch is responsible for the development and implementation of epidemiologic and genetic programs to investigate the cause, prevention, and treatment of neurologic disorders in human populations. Emphasis has been placed on major neurologic diseases in which the diagnoses can be clinically verified to the satisfaction of skilled neurologists. The Branch is unique in being the only unit devoted exclusively to research in the epidemiology of diseases of the nervous system.

Neuroepidemiologic research studies require collaboration of many individuals. However, since there is a severe shortage of available manpower in neuroepidemiology, the Branch has developed an active teaching program for current and future collaborative investigators. A series of six videotapes produced by the Branch are distributed on a loan basis without charge. A textbook, entitled NEUROLOGICAL EPIDEMIOLOGY: PRINCIPLES AND CLINICAL APPLICATIONS, has been prepared. In cooperation with the World Federation of Neurology Research Committee on Neuroepidemiology, a formal course was conducted in Hamburg, West Germany in September, 1985. Another course is planned to be held in Beijing, the People's Republic of China in September, 1986. We are also providing opportunities for fellows to spend from six months to two years working with members of the Branch in order to learn the techniques of neuroepidemiology. During the past year we have had physicians from Ecuador, India, Italy, the People's Republic of China, Costa Rica, Canada, Tunisia, Japan, Ivory Coast, Nigeria, Venezuela, and Israel. Neuroepidemiology was selected as one of the four main themes for the World Congress of Neurology held in Hamburg, West Germany in 1985. These sessions serve as a stimulus for neuroepidemiologic research on a worldwide basis. Finally, current individual and institutional research training grant programs have been expanded to include neuroepidemiology. Institutional grants for training in neuroepidemiology have been awarded to Columbia University, New York, the University of California at Los Angeles, and Temple University, Philadelphia.

Epidemiologic studies have two basic requirements: uniformity and accuracy of data collection. This necessitates

the use of a standardized, internationally accepted classification and coding system. The currently available scheme published by the World Health Organization is seriously deficient with regard to neurologic disorders. The Branch is therefore collaborating with the World Health Organization Neurosciences Program, the World Federation of Neurology, and the American Academy of Neurology to revise this system of classification and improve its usefulness for neuroepidemiologic research. Two members of the Branch were selected to serve on the advisory committee to the World Health Organization to make recommendations for changes in this classification. A draft of the proposed changes has been prepared and has been circulated to neuroscientists from around the world for comments. However, since this new classification will not be available until 1992, the currently available scheme has been modified to make it more suitable for worldwide research in neuroepidemiology. This scheme will be published by the World Health Organization.

Another important problem for the neuroepidemiologist is the enormous cost of maintaining neurologic surveillance on a large number of patients. Therefore, we have attempted to utilize existing registries of neurologic diseases, such as in a study of presenile dementia based on the Israeli National Neurologic Disease Registry. In addition, we have assisted British investigators in organizing information routinely collected through the British National Health Service on all neurologic inpatients in a section of London with a population of 3-1/2 million inhabitants. The utility and accuracy of these data have been demonstrated in a study of the Guillain-Barré syndrome. A similar registry is being organized for the population of northeastern Italy, and another one in Hungary. We also collaborate with the Mayo Clinic in Rochester and utilize their record-linkage system to study neurologic diseases in the population of Rochester, MN.

Epilepsy is a major cause of morbidity and mortality on a world-wide basis. A considerable research effort is devoted by personnel of our Branch to studying this disease. It is our hypothesis that the different types of seizures have different risk factors, and that investigation of a single seizure type increases homogeneity of the case group and, therefore, the likelihood of identifying specific risk factors. Case-control studies were undertaken to determine risk factors for specific types of seizures, utilizing the records-linkage system of Rochester, MN.

In the case-control study of complex partial seizures, the following risk factors were identified: epilepsy or febrile seizures in the mother; small-for-date or pale or blue skin color at birth; neonatal convulsions; cerebral palsy; febrile seizures; head trauma; and viral encephalitis. In the case-control study of absence seizures, only febrile seizures were found to be

significantly associated with absence seizures. The case-control study of tonic-clonic seizures is now in the stage of data analysis.

Studies of the relationship between ease of seizure control and prior duration of untreated epilepsy are being carried out in India and Venezuela. Untreated patients with epilepsy are being identified either through a door-to-door survey or from neurologic clinics. They are then placed on a standard local treatment regimen and their response to treatment monitored. All of these studies are using a uniform protocol.

Another neurologic disease of increasing importance in most of the developed countries, including the U.S., is Alzheimer's disease. This is another area of major research interest to our Branch personnel. Uniform diagnostic criteria are applied to all studies of Alzheimer's disease being conducted in our Branch. The clinical diagnosis is based on the criteria proposed by the NINCDS-Alzheimer's Disease and Related Disorders Association, Inc. work group. Where possible pathological verification of the diagnosis is obtained.

A careful review of the literature on dementia since 1907 has been completed. Special attention has been given to the cases of dementia originally described in Alzheimer's laboratory in Munich (West Germany). Using the United Nations population projections for the 20 year period 1980-2000, the possible effect of demographic trends on senile dementia prevalence in several "developed countries" (United Nations definition) has been studied. The estimated percent increase ranges from very low values in countries that will experience small demographic changes (France, Great Britain, and Sweden) to very high values in nations expected to undergo considerable changes in their elderly population (Italy 39.9%, U.S. 42.0%, and Japan 76.7%).

Four morbidity surveys of Alzheimer's disease are being or have been carried out. One is based on the records-linkage system of the Mayo Clinic which includes the entire population of Rochester, Minnesota. This study will enable us to estimate the incidence of Alzheimer's disease in a well-defined U.S. population. The second investigation utilized a two-stage survey consisting of a questionnaire and clinical examination. This survey has estimated the prevalence of severe dementia in Copiah County, Mississippi, which has equal proportions of black and white Americans. A third survey (in collaboration with the National Institute on Aging) is based on the on-going Honolulu Heart Project. This cohort of people have been followed prospectively for over 20 years and are now in the high risk age group for Alzheimer's disease. These people have been subject to repeated interviews and serum chemistry analyses. These prospectively collected data will be studied as risk factors for Alzheimer's disease. In addition, some members of this cohort

who are all of Japanese origin have come to autopsy. These cases will be intensively studied pathologically to identify the cause of dementia. The fourth survey of Alzheimer's disease has been conducted in Israel based on the nationwide registry of neurologic disease which exists for all Jewish residents of Israel. All cases diagnosed as having "dementia" of any etiology, between 1974 and 1978 were identified and the incidence rate of clinically diagnosed Alzheimer's disease determined based on the population age 60 and younger as well as for the different ethnic groups of this population.

In the study being conducted in Rochester, Minnesota, a neurologist using fixed diagnostic criteria, has reviewed records from all medical facilities serving the residents of Rochester, MN. This made it possible for the first time to determine the incidence of dementia coming to medical attention in a well-defined U.S. population. To confirm the reduced survival of demented patients reported on the basis of individuals hospitalized at specific medical centers, we have examined the survival of all demented individuals identified through this records-linkage study. Dealing with an entire population minimizes any possible selection bias that may be present for a series of patients seen at a particular medical institution. Data from this study are currently being analyzed in detail.

In the study conducted in Copiah County, it was found that for either sex, the prevalence ratios of all severe dementia of the Alzheimer's type were at least as large among blacks as among whites. For either race, the corresponding prevalence ratios were greater in females. For each race and sex, the corresponding prevalence ratios increased with advancing age. Finally, in the population studied, approximately 1% of individuals 40 years old or older had severe dementia. This figure increased to 7% for individuals 80 years old or older. The study in Honolulu is currently in progress. Clinical evidence of dementia has been obtained. Autopsies are being reviewed. The study conducted in Israel found that even among relatively younger individuals, rates increase with age and the risk is slightly greater among women. These findings are consistent with the epidemiologic patterns of Alzheimer's disease in older individuals. Also, a marked difference in the incidence rate of the disease has been found between Jews born in Europe-America (2.9/105) and those from Africa or Asia (1.4/105).

In addition, two case-control studies of Alzheimer's disease patients have been completed and two are in progress to determine risk factors for this disease. The first study uses cases and controls selected from the Rochester, MN population. Past medical records have been utilized to obtain information concerning possible associations between Alzheimer's disease and either medical conditions or surgical procedures. This study has

the advantage that recall bias cannot affect the results of the study since data are being abstracted from medical records. This study is in the advanced stages of data analysis. Three case-control studies of Alzheimer's disease utilizing interview data are being carried out in conjunction with a) the Alzheimer's Disease and Related Disorders Association, in Denver, Colorado, b) the Italian National Research Council, and c) The Burke Rehabilitation Center, White Plains, New York. The study in Denver and the one in Italy have been completed.

The case-control study in Denver has been completed. It was a study of late-onset Alzheimer's disease and was designed to test the hypothesis that risk factors may be different in early- and late-onset Alzheimer's disease. An interesting finding in this study is that in late-onset Alzheimer's disease, family history of dementia is not an important risk factor, thus suggesting that inheritance on a genetic basis may be more important in younger-onset cases than in older-onset cases.

The second case-control study conducted in Italy has also been completed. Two factors were found to be significantly associated with Alzheimer's disease - advanced age of the mother and a family history of dementia. Since the protocol utilized in Italy is similar to the one being used at the Burke Rehabilitation Center, White Plains, New York, international comparisons will be possible. This last case-control study is still in the process of data collection.

Yet another approach will utilize information obtained from clinical examination and combine it with autopsy data, thereby establishing a more definitive diagnosis of Alzheimer's disease. The objective of this study is to highlight the clinical characteristics which are most closely associated with pathologically proven Alzheimer's disease. This should help improve clinical diagnosis.

Epidemiologic studies of stroke have been ongoing by various Branch personnel for many years. The specific questions being currently addressed include: (1) what is the risk of stroke and transient ischemic attacks (TIA) in individuals with heart disease and/or hypertension as compared to the risk in individuals without these conditions; (2) whether the existence of pre-existing heart disease and/or hypertension affects the type of stroke and whether it affects survival following stroke; and (3) whether there is a particular time interval following the onset of heart disease or hypertension during which an individual is at high risk for stroke. In addition, other studies address the issue of the effect of weather on stroke incidence, and a description of autopsy findings for patients dying with stroke in a defined community.

The first study involves a nonconcurrent prospective approach evaluating a cohort of 2,000 elderly individuals. The type of analysis follows the person-years strategy and utilizes life-table methods. This will be followed by an analysis using a time-dependent proportional hazards model to evaluate the individual contributions of hypertension and specific forms of cardiac disease to the risk of both transient ischemic attack and completed ischemic stroke. The investigations of weather variables and autopsy patterns are based on the records-linkage resource for residents of Rochester, MN.

When the case-control approach was applied to data available for the cohort of 2,000 individuals, different patterns of risk factors were demonstrated for TIA and completed ischemic stroke. While hypertension, diabetes mellitus, definite hypertensive heart disease, and valvular heart disease are important risk factors for completed stroke, these disorders have substantially less effect on the subsequent risk of TIA. When these data were analyzed in the format of a prospective study, it was possible to calculate the absolute risk of stroke as a function of the presence or absence of specific forms of cardiovascular disease. The following forms of cardiovascular disease yielded the highest ischemic stroke incidence rates (given in cases/1,000/year): myocardial infarction (15.5); congestive heart failure (20.5); and TIA (42.0). In considering risk factors for TIA, both angina/coronary insufficiency and congestive heart failure yielded the highest rates (10.4 and 10.9, respectively). Once etiologic precursors of stroke have been identified, medical intervention before the occurrence of long-lasting disability requires that there be an interval of time between the onset of the risk factor and the development of completed stroke. Analysis of data from this nonconcurrent prospective study revealed that those developing borderline hypertension, valvular heart disease, or ischemic heart disease remained stroke-free for the first 1-1/2 years after the first occurrence of each specific form of cardiovascular disease. This finding implies that there is an interval of time following the onset of these conditions when it may be possible to intervene medically to reduce the risk of stroke. Based on the time-dependent proportional hazards model, hypertension and transient ischemic attacks had the highest relative risks.

Other investigations in the area of stroke involve the careful analysis of unusual patterns of cerebrovascular disease (e.g., more than 20 TIA's/day). The study of weather variables and stroke revealed that temperature has no effect on stroke incidence.

Although death certificate data are limited by possible misdiagnosis, incomplete case ascertainment, errors in coding, etc., detailed mortality information on some neurologic diseases for the entire U.S. is not available. Analysis of mortality data



can be particularly useful for some neurologic diseases because these may contribute to death indirectly. Since there are no uniform criteria for what constitutes the underlying cause of death in patients, it is important to examine all deaths in which a disease is listed as an underlying, immediate, associated, or a contributory cause of death to get more complete information about the relationship between the disease and death. Association of diseases occurring at the time of death was also studied for all deaths occurring in the U.S. for many neurologic diseases. Diseases occurring together may provide important information in the search for etiology of diseases. Such detailed analysis of mortality data have been done for Alzheimer's disease and related diagnoses, motor neuron disease, hereditary ataxia, and Down's syndrome, for 1971-1978. The overall patterns which have emerged have been useful in evaluating trends over time and in formulating etiologic hypotheses.

All death certificates for the entire U.S. for the years 1971 and 1973 through 1978 were searched for the diagnosis of senile and presenile dementia and senility. Age-, race-, and sex-specific mortality rates for deaths due to and deaths with these dementias were calculated. Time trends in the age-adjusted mortality rates between 1971 and 1978 were also calculated. To determine which conditions may be associated with reduced survival in patients with Alzheimer's disease, all death certificates in the United States for 1978 on which "senile and presenile" dementia was mentioned have been studied. Each case was compared with two control deaths.

The age-adjusted mortality rates for two types of dementia (one being senile and presenile dementia and the other senility) were higher for deaths with these conditions than due to them. Other diseases were listed as the underlying cause of death in most patients who died with dementia. Between 1971 and 1978, there was an increase in the age-adjusted mortality rates for senile and presenile dementia but a decline for senility. Age-specific mortality rates for both types of dementia increased exponentially with age, with no evidence of bimodality. Conditions associated with reduced survival in patients with Alzheimer's disease were: infections, trauma, nutritional deficiency, chronic ulcer of skin, foreign body in pharynx, cataract, glaucoma, blindness, deafness, Parkinson's disease, and epilepsy. There seem to be many preventable and treatable disorders in patients with senile and presenile dementia.

Similar analyses are being conducted for motor neuron disease, Down's syndrome, spina bifida, and anencephaly.

Many different approaches have been utilized to study tumors of the nervous system. Descriptive epidemiologic techniques were applied to data obtained from tumor registries around the world.

Wherever possible, cases were reviewed by the Branch's staff. New analytic techniques for studies of multiple primary cancers were devised. A case-control study is being done to estimate the association between putative risk factors and histologically confirmed grade III and IV astrocytomas in adults.

On the basis of the descriptive studies, two patterns of age-specific incidence emerged. Analyses of most population-based data worldwide revealed a small childhood peak, followed by a later peak between ages 50 and 80. Data for Rochester, MN, however, showed the childhood peak, followed by an increasing incidence rate with increasing age. Careful study of this discrepancy showed 1) that the greater percentage of cases first diagnosed at autopsy in Rochester accounted in large part for this difference, and 2) that a substantial number of brain tumors remain undiagnosed in the elderly during life. Studies are currently underway to evaluate the role of computerized tomography in the diagnosis of brain tumors and to explain the recent increase in the incidence of pituitary tumors among women of childbearing age. In addition, racial differentials in the frequency of certain intracranial tumors (meningiomas and pituitary adenomas) are being examined. Investigations of the relationship between intracranial neoplasms and extracranial tumors have been especially rewarding. An association was found between the occurrence of breast cancer and meningioma in women. This result raises interesting etiologic possibilities when considered with other evidence: 1) meningioma is the only common intracranial neoplasm with a higher incidence in females; 2) the abrupt clinical appearance or enlargement of this tumor during pregnancy has been described; and 3) the finding of estrogen receptor protein in meningioma has been reported. This has led to further work concerning estrogen receptors in other tumors such as malignant melanoma involving the nervous system.

There is continuing controversy regarding racial differences in the occurrence of major neurologic diseases. Thus, a study was conducted in Copiah County, Mississippi to specifically address this question. A strategy was developed which eliminated the requirement that persons must have entered the health care system for detection of disease. The basis of the investigation was a door-to-door survey which utilized a detailed questionnaire inquiring not only about diagnoses, but also about signs and symptoms suggestive of neurologic dysfunction. Over 99% of the households agreed to the interview. Those household members suspected of having one of the disorders of interest were then asked to have a neurologic examination conducted by a senior, board-certified neurologist. This study showed that severe dementia of the Alzheimer's type is almost as common among black Americans as it is among white Americans. The prevalence ratio of Parkinson's disease is similar among blacks and whites residing in the same county in the U.S., whereas the prevalence of Parkinson's disease among blacks in West Africa is much

lower. These findings suggest the importance of environmental factors in the etiology of Parkinson's disease.

Data from different parts of the world is often not directly comparable because of the use of different definitions of disease, different methodology to estimate disease frequency, and results are affected by availability of and access to experts in neurology. A protocol developed by the Branch in collaboration with the World Health Organization has eliminated these biases. The uniform protocol which is being used to estimate the prevalence of major neurologic disorders in many parts of the world, will enable international comparisons.

Pilot studies in Nigeria indicate that migraine is at least as common in rural black Africa as it is in urban populations of Western Europe. Furthermore, the prevalence of epilepsy is higher in rural Nigeria than reported for developed countries. In the People's Republic of China, the prevalence ratio of cerebrovascular disease is greater than anywhere else in the world where this disease has been studied.

The clinical neurogenetics component of the Branch involves three areas: 1) genetic-epidemiologic studies of movement disorders (e.g., the dystonias); 2) genetic-epidemiologic studies of multifactorial neurologic disorders (e.g., Parkinson's disease, Alzheimer's disease, and multiple sclerosis); and 3) genetic and biochemical studies of hereditary nervous system tumors.

Included among the disorders of movement such as the choreas, the dystonias, and tic syndromes are a number of discrete diseases which are due to a single gene mutation. Examples of mutations producing autosomal dominant traits are Huntington's chorea and one form of torsion dystonia. Examples of mutations leading to autosomal recessive traits are Lafora type myoclonic epilepsy, the newly described Baltic type myoclonus epilepsy, and the type of torsion dystonia probably responsible for most cases of dystonia in the Jewish population. Studies are being conducted to 1) uncover additional specific diseases within general movement disorder syndromes; 2) contribute to the understanding of their underlying biochemical basis; 3) determine the most effective treatment for each disorder; and 4) suggest guidelines for counseling individual family members.

Initially, families with members exhibiting a particular syndrome undergo detailed clinical evaluation. Extensive genealogical data are then analyzed in conjunction with clinical observations and relevant laboratory studies. A nosologic classification is prepared. Promising biochemical leads, particularly involving neurotransmitter metabolism, are explored in collaboration with established investigators. Simultaneously, existing treatment programs are evaluated, and where indicated,

there are therapeutic trials of new agents. Based on low CSF biopterin in a form of familial dystonia, biopterin has been administered intravenously to 10 patients resulting in transient improvement in several.

Together with colleagues at Johns Hopkins Hospital we have reported the first example of Munchausen Syndrome presenting as torsion dystonia. The patient underwent brain surgery, tracheostomy, and feeding gastrostomy before she told us of the factitious basis for her dystonia-like symptoms.

Our goal is to determine the relative genetic and environmental contribution to multifactorial diseases. Focus is on likely biochemical or immunogenetic abnormalities suggesting a specific genetic role coupled with the search for environmental risk factors. Approaches and techniques used are from multiple disciplines including clinical, population, and biochemical genetics coupled with descriptive and analytical epidemiology. Selected populations including families with multiple members or twin pairs affected with the disease are studied in depth. Unaffected family members, unaffected twins, and spouses serve as controls.

What may be a new adult onset leukodystrophy but associated with major autonomic system disturbance and diagnosable by CT and MRI scan has been mistaken for MS in at least 20 members of one kindred. Derangement of the autonomic nervous system is often seen early in the course and when recognized, serves to distinguish this single gene disorder clinically from multiple sclerosis of the chronic progressive type. Extensive studies were performed on 9 at-risk individuals, 8 offspring, ages 23 to 32, and one 58-year-old sibling. Evoked potentials (BAEP's, VEP's, and SEP's) and physiologic autonomic testing were normal in all nine. A 32-year-old asymptomatic male had subtle pyramidal findings on examination with CT/MRI changes in the frontoparietal and cerebellar white matter, consistent with an early stage of the disorder. Another 30-year-old male had a site of increased signal intensity in the parietal-occipital area on MRI. A long history of constipation and urinary frequency was elicited in a 25-year-old female with normal neuroradiologic evaluation.

Sixty-two twin pairs with Parkinson's disease have now been evaluated. Among 43 monozygotic pairs, there is only one definite concordant pair and there are no definitely concordant pairs among 19 dizygotic pairs, suggesting that the genetic contribution in Parkinson's disease is not the decisive factor in twins. There appears to be a lifelong personality difference between affected and unaffected twins, the affected twin being less outgoing. Smoking is also less frequent in the affected twins, but this may reflect personality difference. A simple, novel, etiologic theory involving initial neuron number has been suggested based on these findings.

The initial phase of a multidisciplinary study of Alzheimer's disease occurring in 22 twin pairs has been completed. Of 17 monozygotic pairs, 7 were concordant. The average duration of disease in the affected member of the 10 discordant pairs was 8 years with a range of 5 to 10 years. Of 5 dizygotic twin pairs, 2 were concordant. The duration of disease in the 3 discordant pairs was 10 years, with a range of 6 to 15 years. Autopsy verification of diagnosis was made in 4 affected twins.

There are at least ten genetically determined syndromes which include as one of their chief manifestations tumors of the nervous system. Neurofibromatosis and tuberous sclerosis are among the more common examples. The objective of another project is to 1) document additional hereditary traits which can cause such neoplasms; 2) add information to the clinical description and natural history of such traits; 3) suggest effective means of early diagnosis; 4) evaluate various modes of treatment; and 5) develop methods of preclinical detection and screening. In families with multiple individuals affected with the same rare tumor of the nervous system, members undergo clinical, genetic, and psychological evaluation. Appropriate physiologic and biochemical studies are carried out in collaboration with laboratory investigators.

We have documented the existence of a distinct form of neurofibromatosis, the hallmark of which is bilateral acoustic neuroma, in addition to the type described by von Recklinghausen. Currently, we are evaluating the usefulness of standard audiologic studies, acoustic reflex decay, brain stem auditory-evoked potential, CT scan and nuclear magnetic resonance in screening at-risk individuals, establishing a diagnosis, and managing those with known bilateral acoustic neuromas. Preliminary results suggest brain stem auditory-evoked potential and acoustic reflex decay are most useful for screening and for monitoring those with small, slowly growing tumors. An additional screening approach we suggest consists of bio-ophthalmoscopic study of the lens. Six of 23 patients exhibited posterior subcapsular opacities which in several instances antedated audiologic or vestibular changes.

Mental retardation, attention deficit disorder, and learning disabilities are considered to be frequent features of von Recklinghausen's neurofibromatosis. However, there are few reports of formal neuropsychological testing of these individuals and none involving rigorous selection of controls. A pilot study evaluating neurological and cognitive function in neurofibromatosis has been performed. Following local announcements, thirteen sibling pairs between the ages of 6 and 26, one affected with neurofibromatosis and one unaffected, were selected. By design, none of the affected individuals had evidence of central nervous system pathology.

The neurofibromatosis cases scored significantly higher in number of subtle neurological abnormalities ( $p < 0.01$ ) and significantly lower in Full Scale IQ ( $p < 0.01$ ) than their unaffected siblings. Their IQ scores were not clustered at the lower end of the scale, but rather, there was a consistent shift downward in their IQ distribution when compared with their siblings. In addition, a deficit in visual-spatial orientation was present in cases ( $p < 0.025$ ). No significant excess of low cognitive function, attention deficit disorder, or learning disabilities were found.

In the field of pediatric neuroepidemiology a study of mental retardation is being conducted in Rochester, Minnesota, using their records-linkage system. The objectives of this study are to determine the incidence of mental retardation, temporal trends in incidence and risk factors for mental retardation in this population.

Though emphasis is placed on research on the major diseases of the nervous system, other diseases which may be less frequent or less debilitating but important in terms of pathogenesis or clues to disease etiology, are also under investigation in our Branch.

In collaboration with other Governmental agencies (Centers for Disease Control), other Governments (Colombia, Republic of Seychelles), International Organizations (World Health Organization), and Universities (Mayo Medical School) methods have been developed to investigate less common neurologic disorders. Several such studies have been completed. Current projects include investigation of space/time clusters of neurologic disease (with the Centers for Disease Control), clusters of multiple sclerosis (with the Multiple Sclerosis Society), a study of myasthenia gravis and multiple sclerosis in the same patient (with the Mayo Clinic), an investigation of neurologic disorders during pregnancy and the postpartum period (with the Mayo Clinic), an investigation of pseudotumor cerebri diagnosed at the University of Illinois, Chicago, a prevalence and case-control study of normal pressure hydrocephalus (with VA Medical Center, Shreveport, Louisiana), a prevalence and case-control study of progressive supranuclear palsy (with University of Medicine and Dentistry of New Jersey), descriptive and analytic studies of spastic paraparesis in different parts of the world, and a study of risk factors for cerebral malaria.

Headaches are an important cause of disability in the general population. However, because of problems with definitions and methodology of survey, reliable population based data on the prevalence of headaches is not available. Surveys have been conducted in Mexico and the People's Republic of China to estimate the prevalence of headaches. These studies have utilized a uniform protocol, thus international comparisons will

be possible. The study of headaches in China showed that the prevalence of "incapacitating headaches" was about twice as common in women compared to men and also about twice as common in the urban areas compared to rural areas.

The study of pseudotumor cerebri showed that low protein in the cerebrospinal fluid (CSF) often reported in these patients is related to high CSF pressure. This study documented an inverse linear relationship between CSF protein content and CSF opening pressure at the time of lumbar puncture. Further studies are being conducted to elucidate the natural history of pseudotumor cerebri using MRI scans.

A special study of spastic paraparesis in Tumaco, Colombia (along the southern Pacific coast of Colombia) was carried out. The clinical and epidemiologic features of the disease were characterized and special case-control studies to address etiologic factors were initiated. In a special study of tropical spastic paraparesis in the Seychelles Islands, we discovered a strong association between this disease and antibodies to human T-lymphotropic virus-Type I (HTLV-I).

In collaboration with the U.S. Army, a retrospective cohort approach will be used to study the problem of cerebral malaria. A cohort of individuals who served in the military in Viet Nam from 1968-1970, will be identified and those who can be reached will be examined by questionnaire, physical examination, and psychometric testing.

Development of new neurologic studies requires thorough historic and methodologic reviews of prior investigations. These yield important unexplored etiologic clues that may be investigated using current technology. Major emphasis has been given to: cerebrovascular disease; otitis media; inherited ataxias; Huntington's disease; febrile seizures; Tourette's syndrome; peripheral neuropathy; neurologic disease in the elderly; controlled therapeutic trials of motor neuron disease; epilepsy; descriptive, analytic, and experimental methods in neuroepidemiology; statistical methods for calculating confidence intervals, procedures for neuroepidemiologic investigations in developing countries; and epidemiologic studies of Alzheimer's disease; myasthenia gravis; and cerebral malaria.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 NS 01924-16 NEB</b>
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Clinical, Genetic, Pathophysiologic Study of Hereditary Movement Disorders</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; padding: 5px 0;"> <span><b>Roswell Eldridge, M.D.,</b></span> <span><b>Medical Geneticist,</b></span> <span><b>NEB, IRP, NINCDS</b></span> </div>		
COOPERATING UNITS (if any) <b>ET, IRP, NINCDS: HE, NHLBI: LCS, DCBR, NIMH</b>		
LAB/BRANCH <b>Neuroepidemiology Branch, Intramural Research Program</b>		
SECTION		
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, Maryland 20892</b>		
TOTAL MAN-YEARS: <div style="border: 1px solid black; text-align: center; width: 100px; margin: 0 auto;">0.3</div>	PROFESSIONAL: <div style="border: 1px solid black; text-align: center; width: 100px; margin: 0 auto;">0.3</div>	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In this project, we seek to 1) clarify and expand the nosology of the hereditary movement disorders; 2) contribute to the understanding of the underlying biochemical basis; 3) determine the most effective treatment for each disorder; and 4) suggest guidelines for counseling individuals at risk. General syndromes under study include the dystonias, tic disorders, blepharospasm, and myoclonus. Approaches include standard epidemiologic and clinical genetic studies together with collaborative efforts in evaluating the role of neurotransmitters such as dopamine, their precursors, and metabolites, and their necessary cofactors.</p> <p>Collaborative studies are underway with personnel in LCS, DCBR, NIMH and HE, NHLBI to explain our earlier observations of altered dopamine beta hydroxylase and norepinephrine levels in blood and biopterin in CSF in a genetic subset of dystonia patients. Members of selected families are admitted to the Clinical Center, NIH, for trial of several new pharmacological agents.</p> <p>Biopterin administered intravenously has led to acute benefit in one form of generalized dystonia.</p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01927-16 NEB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Genetic, Pathophysiologic Study of Hereditary Nervous System Tumors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Roswell Eldridge, M.D.

Medical Geneticist,

NEB, IRP, NINCDS

COOPERATING UNITS (if any) OP, CC: SN, IRP, NINCDS: Division of Medical Genetics, Dept. of Pediatrics, Children's Hospital National Medical Center; Dept. of Neurosurgery, Massachusetts General Hospital, Boston, MA

## LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

## SECTION

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL:

0.3

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project we seek to define and classify hereditary tumors of the nervous system such as occur in neurofibromatosis; to add to the clinical description and natural history of these diseases; to suggest methods for early diagnosis; to evaluate present modes of treatment; and to develop methods for preclinical detection and screening.

Our studies have led to the recognition of at least two distinct genetic forms of neurofibromatosis: 1) the classical form as described by von Recklinghausen, and 2) a form in which bilateral acoustic neuromas are the hallmark. Efforts in the latter have been directed at improving and simplifying screening of high-risk individuals, confirming diagnosis, and establishing criteria for intervention. Audiologic studies, including evaluation of auditory-evoked response and acoustic reflex decay, are useful means for early documentation and monitoring of acoustic neuromas. We have recently found that presenile lens opacities or cataracts occur in about 50% of such patients.

In our first major study involving neurofibromatosis of the von Recklinghausen type, a multi-disciplinary project is nearing completion to evaluate neurologic and cognitive status in these patients compared to their unaffected sibs. We plan to initiate gene-linkage studies in neurofibromatosis, so successful in Huntington disease.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02167-12 NEB
PERIOD COVERED <u>October 1, 1985 through September 30, 1986</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Genetic Epidemiology Studies in MS and Other Multifactorial Neurologic Disorders</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between;"> <span>Roswell Eldridge, M.D.</span> <span>Medical Geneticist,</span> <span>NEB, IRP, NINCDS</span> </div>		
COOPERATING UNITS (if any)  NI, IRP and OBFS, OD, NINCDS: M CN NIMH: Department of Neurology, Monmouth Medical Center, Monmouth, NJ		
LAB/BRANCH <u>Neuroepidemiology Branch, Intramural Research Program</u>		
SECTION		
INSTITUTE AND LOCATION <u>NINCDS, NIH, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS: <div style="text-align: center;">0.4</div>	PROFESSIONAL: <div style="text-align: center;">0.4</div>	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input checked="" type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>In this project we are coupling genetic and environmental studies in selected families and twin pairs with disorders such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease, in an effort to distinguish specific contributing factors.</p> <p>A multi-disciplinary twin study of Parkinson's disease has led to formulation of an etiologic theory we term the "initial neuron number" hypothesis. Since neurons in the substantia nigra are not known to regenerate but rather appear to die off at a constant rate during adulthood, starting life with a reduced number of these critical neurons may be one predisposing factor to eventual development of the disorder.</p> <p>A study similar in design involving twins with dementia of the Alzheimer's types also indicates environmental factors must be involved in some forms of the disorder.</p> <p>An autosomal dominant, hereditary leukoencephalopathy simulating MS with onset at about age 35 is under study in a kindred with over 20 affected. Derangement of the autonomic nervous system is often seen early in the course and when recognized clinically, serves to distinguish this single gene disorder from multiple sclerosis. Computerized tomographic scan changes of the brain are characteristic, even in early cases.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02240-10 NEB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiology of Dementia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Bruce S. Schoenberg, M.D.,	Dr.P.H. Chief,	NEB, IRP, NINCDS
Vijay Chandra, M.D.,	Ph.D. Medical Officer,	NEB, IRP, NINCDS
Luigi Amaducci, M.D.	Visiting Scientist,	NEB, IRP, NINCDS
Walter Rocca, M.D.,	M.P.H. Visiting Scientist,	NEB, IRP, NINCDS
Threse Treves, M.D.	Visiting Scientist,	NEB, IRP, NINCDS

COOPERATING UNITS (if any)

Epidemiology, Demography, and Biometry, NIA; W. Massey, M.D., Duke Univ.; E. Kokman, M.D., and J.P. Whisnant, M.D., Mayo Clinic; B. Jordan, Columbia University, NY

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Population-based estimates of the incidence, prevalence, morbidity and mortality of Alzheimer's disease for the U.S. have not been available. A study has recently been completed for the entire population of Rochester, Minnesota where the incidence of Alzheimer's disease has been estimated. This is the first study of its kind in the U.S. Another study to estimate the prevalence of "severe dementia" in a population with equal proportions of black and white Americans has also been completed and published. National mortality data for the entire U.S. for the period 1971, 1973-1978 for Alzheimer's disease and related diagnoses has also been analyzed for the first time and recently published.

Analytic studies to determine risk factors for Alzheimer's disease are being conducted. A case-control study of late-onset Alzheimer's disease has been completed in Denver, Colorado. Another case-control study of Alzheimer's disease is in progress in White Plains, New York. A multi-center case-control study has also been completed in Italy. Since this study utilized a similar protocol to that used in the U.S., international comparisons are possible.

A historical review of the distinction between "Alzheimer's disease" and "senile dementia" has been done. Projections for the prevalence of senile dementia in the year 2000 for several developed countries have been developed. An attempt has been made to evaluate and improve instruments used in case-control studies of dementia, and also attempts to improve the clinical diagnosis of Alzheimer's disease are being made.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02241-10 NEB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Epidemiology of Cerebrovascular Disease in Adults		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between;"> <div style="width: 60%;">           Bruce S. Schoenberg, M.D., Dr.P.H. Chief,            Patricia Davis, M.D. Guest Researcher            James Dambrosia, Ph.D. Chief,         </div> <div style="width: 35%;">           NEB, IRP, NINCDS            NEB, IRP, NINCDS            BFSB, IRP, NINCDS         </div> </div>		
COOPERATING UNITS (if any) J.P. Whisnant, M.D., Mayo Clinic; D.G. Schoenberg, M.S., Bethesda, Maryland, A. Lilienfeld, M.D., Johns Hopkins University		
LAB/BRANCH Neuroepidemiology Branch, Intramural Research Program		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.6	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>           This investigation is aimed 1) at evaluating the effect of heart disease and hypertension as potentially treatable precursors of completed stroke and transient ischemic attacks; 2) at documenting unusual patterns of cerebrovascular disease; 3) at determining the autopsy patterns for patients dying with cerebrovascular disease in defined community; and 4) at examining if weather parameters have any effect on stroke incidence.         </p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02243-10 NEB									
PERIOD COVERED October 1, 1985 through September 30, 1986											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pediatric Neuroepidemiology											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 60%;">Bruce S. Schoenberg, M.D., Dr.P.H. Chief,</td> <td style="width: 40%;">NEB, IRP, NINCDS</td> </tr> <tr> <td>Vijay Chandra, M.D., Ph.D. Medical Officer,</td> <td>NEB, IRP, NINCDS</td> </tr> <tr> <td>Lawrence Lavine, M.D. Medical Officer,</td> <td>NEB, IRP, NINCDS</td> </tr> </table>			Bruce S. Schoenberg, M.D., Dr.P.H. Chief,	NEB, IRP, NINCDS	Vijay Chandra, M.D., Ph.D. Medical Officer,	NEB, IRP, NINCDS	Lawrence Lavine, M.D. Medical Officer,	NEB, IRP, NINCDS			
Bruce S. Schoenberg, M.D., Dr.P.H. Chief,	NEB, IRP, NINCDS										
Vijay Chandra, M.D., Ph.D. Medical Officer,	NEB, IRP, NINCDS										
Lawrence Lavine, M.D. Medical Officer,	NEB, IRP, NINCDS										
COOPERATING UNITS (if any) D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland; J.F. Mellinger, M.D., M.R. Gomez, M.D., L.T. Kurland, M.D., Dr.P.H. and R.V. Groover, M.D., Dept. of Neurology, Mayo Clinic; L.L. Salkowicz; P. Gunderson, Ph.D., Minnesota Dept. of Health ; *											
LAB/BRANCH Neuroepidemiology Branch, Intramural Research Program											
SECTION											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892											
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>A study of mental retardation is being conducted in Rochester, Minnesota, using their records-linkage system. The objectives of this study are to determine the incidence of mental retardation, temporal trends in incidence and risk factors for mental retardation in this population.</p>											
<hr style="border-top: 1px dashed black;"/> <p>*Continued:</p> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">M. Cruz, M.D.,</td> <td style="width: 33%;">Mayo Clinic,</td> <td style="width: 33%;">Rochester, MN</td> </tr> <tr> <td>F. Baker, M.D.,</td> <td>USPHS Epidemiology</td> <td></td> </tr> <tr> <td></td> <td>Training Program</td> <td>Bethesda, MD</td> </tr> </table>			M. Cruz, M.D.,	Mayo Clinic,	Rochester, MN	F. Baker, M.D.,	USPHS Epidemiology			Training Program	Bethesda, MD
M. Cruz, M.D.,	Mayo Clinic,	Rochester, MN									
F. Baker, M.D.,	USPHS Epidemiology										
	Training Program	Bethesda, MD									

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 NS 02297-10 NEB</b>		
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>				
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Mortality from Neurologic Disorders: National and International Comparisons</b>				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <b>Bruce S. Schoenberg, M.D., Dr.P.H. Chief,</b>  <b>Vijay Chandra, M.D., Ph.D. Medical Officer,</b>  <b>Maurizio Leone, M.D. Guest Researcher,</b>  <b>Hisaharu Suzuki, M.D. Guest Researcher,</b> </td> <td style="width: 50%; vertical-align: top;"> <b>NEB, IRP, NINCDS</b>  <b>NEB, IRP, NINCDS</b>  <b>NEB, IRP, NINCDS</b>  <b>NEB, IRP, NINCDS</b> </td> </tr> </table>			<b>Bruce S. Schoenberg, M.D., Dr.P.H. Chief,</b> <b>Vijay Chandra, M.D., Ph.D. Medical Officer,</b> <b>Maurizio Leone, M.D. Guest Researcher,</b> <b>Hisaharu Suzuki, M.D. Guest Researcher,</b>	<b>NEB, IRP, NINCDS</b> <b>NEB, IRP, NINCDS</b> <b>NEB, IRP, NINCDS</b> <b>NEB, IRP, NINCDS</b>
<b>Bruce S. Schoenberg, M.D., Dr.P.H. Chief,</b> <b>Vijay Chandra, M.D., Ph.D. Medical Officer,</b> <b>Maurizio Leone, M.D. Guest Researcher,</b> <b>Hisaharu Suzuki, M.D. Guest Researcher,</b>	<b>NEB, IRP, NINCDS</b> <b>NEB, IRP, NINCDS</b> <b>NEB, IRP, NINCDS</b> <b>NEB, IRP, NINCDS</b>			
COOPERATING UNITS (if any) <b>W. Massey, M.D., Duke University; D.G. Schoenberg, M.S., Bethesda, Maryland</b>				
LAB/BRANCH <b>Neuroepidemiology Branch, Intramural Research Program</b>				
SECTION 				
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, Maryland 20892</b>				
TOTAL MAN-YEARS: <div style="text-align: center;">1.7</div>	PROFESSIONAL: <div style="text-align: center;">1.7</div>	OTHER: 		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews				
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>Although death certificate data are limited by possible misdiagnosis, incomplete case ascertainment, errors in coding, etc., detailed mortality information on some neurologic diseases for the entire U.S. is not available. Analysis of mortality data can be particularly useful for some neurologic diseases because these may contribute to death indirectly. Since there are no uniform criteria for what constitutes the underlying cause of death in patients, it is important to examine all deaths in which a disease is listed as an underlying, immediate, associated, or contributory cause of death to get more complete information about the relationship between the disease and death. Association of diseases occurring at the time of death was also studied for all deaths occurring in the U.S. for many neurologic diseases. Diseases occurring together may provide important information in the search for etiology of diseases. Such detailed analysis of mortality data have been done for Alzheimer's disease and related diagnosis, motor neuron disease, hereditary ataxia, and Down's syndrome, for 1971, 1973-1978. The overall patterns which have emerged have been useful in evaluating trends over time and in formulating etiologic hypotheses.</p>				

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02299-10 NEB

## PERIOD COVERED

October 1, 1985 through September 30, 1986

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reviews of Epidemiology Aspects of Neurologic Disease

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Bruce S. Schoenberg, M.D., Dr.P.H.	Chief,	NEB, IRP, NINCDS
Luigi Amaducci, M.D.	Visiting Scientist,	NEB, IRP, NINCDS
Walter A. Rocca, M.D., M.P.H.	Visiting Associate,	NEB, IRP, NINCDS
Patricia Davis, M.D.	Guest Researcher,	NEB, IRP, NINCDS
Therese Treves, M.D.	Visiting Scientist,	NEB, IRP, NINCDS
Lawrence Lavine, M.D.	Medical Officer,	NEB, IRP, NINCDS

## COOPERATING UNITS (if any)

W. Massey, M.D., Duke University; D. Schoenberg, M.S., Bethesda, Maryland;  
N. Bharucha, M.D., Bombay, India

## LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

## SECTION

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.7

## PROFESSIONAL:

0.7

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Development of new neurologic studies requires thorough historic and methodologic reviews of prior investigations. These yield important unexplored etiologic clues that may be investigated using current technology. Major emphasis has been given to: cerebrovascular disease; otitis media; inherited ataxias; Huntington's disease; febrile seizures; Tourette's syndrome; peripheral neuropathy; neurologic disease in the elderly; controlled therapeutic trials of motor neuron disease; epilepsy; descriptive, analytic, and experimental methods in neuroepidemiology; statistical methods for calculating confidence intervals; procedures for neuroepidemiologic investigations in developing countries; and epidemiologic studies of Alzheimer's disease; myasthenia gravis; and cerebral malaria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02300-10 NEB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Clinical Course and Medical Care for Neurologic Disorders</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  Bruce S. Schoenberg, M.D., Dr.P.H. Chief, <span style="float: right;">NEB, IRP, NINCDS</span>		
COOPERATING UNITS (if any)  J.P. Whisnant, M.D., Department of Neurology, Mayo Clinic, Rochester, MN		
LAB/BRANCH Neuroepidemiology Branch, Intramural Research Program		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.1	PROFESSIONAL: 0.1	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>The study uses a review and abstraction of data from records for a selected group of neurological disorders. It obtains the items of data necessary to determine onset of the disorder, duration, date and cause of death, or current status. These data will be used to construct modified life tables to estimate the expectation of life after diagnosis, the survival curve, and morbidity and severity estimates. It will also include analysis of type and duration of medical care received by patients with neurologic disorders derived from a well-defined population.</p>		



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02301-10 NEB									
PERIOD COVERED October 1, 1985 through September 30, 1986											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Collaborative Studies of Less Common or Less Debilitating Neurologic Disorders											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 60%;">Bruce S. Schoenberg, M.D., Dr.P.H. Chief,</td> <td>NEB, IRP, NINCDS</td> </tr> <tr> <td>Lawrence Lavine, M.D. Medical Officer,</td> <td>NEB, IRP, NINCDS</td> </tr> <tr> <td>Patricia Davis, M.D. Visiting Scientist,</td> <td>NEB, IRP, NINCDS</td> </tr> </table>			Bruce S. Schoenberg, M.D., Dr.P.H. Chief,	NEB, IRP, NINCDS	Lawrence Lavine, M.D. Medical Officer,	NEB, IRP, NINCDS	Patricia Davis, M.D. Visiting Scientist,	NEB, IRP, NINCDS			
Bruce S. Schoenberg, M.D., Dr.P.H. Chief,	NEB, IRP, NINCDS										
Lawrence Lavine, M.D. Medical Officer,	NEB, IRP, NINCDS										
Patricia Davis, M.D. Visiting Scientist,	NEB, IRP, NINCDS										
COOPERATING UNITS (if any) M. Zack, M.D., Atlanta, Georgia; Neurosciences Program, WHO, Geneva, Switzerland; D. Duane, M.D., B. Sandok, M.D., Mayo Clinic; G. Roman, Lubbock, Texas; P.S. Spencer, Albert Einstein College of Medicine, New York *											
LAB/BRANCH Neuroepidemiology Branch, Intramural Research Program											
SECTION											
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892											
TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A number of collaborative efforts involve the investigation of the characteristics of unusual or less debilitating (e.g., headache) neurologic disease phenomena. Unusual associations or space/time clusters of neurologic disorders may provide leads to etiology or therapy. These may be tested through more formal approaches.</p> <p>A study of over 100 cases of pseudotumor cerebri has been completed. This study documented an inverse linear relationship between cerebrospinal fluid (CSF) opening pressure and CSF protein content.</p> <p>Descriptive and analytic studies of spastic paraparesis are being conducted in Columbia, India, the Seychelles Islands, and the West Indies.</p> <p>A retrospective cohort approach is being used to investigate the sequelae of cerebral malaria due to infection with Plasmodium Falciparum.</p>											
----- *Continued: <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">R. Duviosin, M.D.</td> <td style="width: 33%;">Univ. of Medicine &amp; Dentistry of New Jersey</td> <td style="width: 33%;">New Brunswick, NJ</td> </tr> <tr> <td>L. Golbe, M.D.</td> <td style="text-align: center;">" " "</td> <td style="text-align: center;">" " "</td> </tr> <tr> <td>P. Kark, M.D.</td> <td>V.A. Medical Center</td> <td>Shreveport, LA</td> </tr> </table>			R. Duviosin, M.D.	Univ. of Medicine & Dentistry of New Jersey	New Brunswick, NJ	L. Golbe, M.D.	" " "	" " "	P. Kark, M.D.	V.A. Medical Center	Shreveport, LA
R. Duviosin, M.D.	Univ. of Medicine & Dentistry of New Jersey	New Brunswick, NJ									
L. Golbe, M.D.	" " "	" " "									
P. Kark, M.D.	V.A. Medical Center	Shreveport, LA									

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02305-10 NEB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Epidemiology of Intracranial Neoplasms		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <span>Bruce S. Schoenberg, M.D., Dr.P.H. Chief,</span> <span>NEB, IRP, NINCDS</span> </div>		
COOPERATING UNITS (if any) B.W. Christine, M.D., M.P.H., Connecticut State Department of Health; J.P. Whisnant, M.D., and R.J. Campbell, M.D., Mayo Clinic; L. Mahalak, M.D., Jackson, MS; A. Heck, M.D., Univ. Of TN; R. Simon, M.D., Berkley, CA; B. Jordan, B.A., Harvard Medical School		
LAB/BRANCH Neuroepidemiology Branch, Intramural Research Program		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.3	PROFESSIONAL 0.3	OTHER
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="margin-top: 10px;"> <p>The Branch has conducted extensive investigations on the descriptive epidemiology of primary intracranial neoplasms using data from population-based registries worldwide. Analytic studies were carried out to investigate the relationship between intracranial neoplasms and tumors occurring at other sites. These studies included careful review of tumor nomenclature, disease definitions, and survey strategies. A case-control study of glioblastoma multiforme is now being conducted.</p> </div>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02307-10 NEB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Educational Resources in Neurological Epidemiology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  Bruce S. Schoenberg, M.D., Dr.P.H. Chief, <span style="float: right;">NEB, IRP, NINCDS</span>		
COOPERATING UNITS (if any) D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland		
LAB/BRANCH Neuroepidemiology Branch, Intramural Research Program		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 0.1	PROFESSIONAL: 0.1	OTHER
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided )  Because there is severe shortage of available manpower in neuroepidemiology, the Branch has developed an active teaching program for current and future collaborative investigators. A series of six video tapes produced by the Branch are distributed on a loan basis without charge. A textbook, entitled NEUROLOGICAL EPIDEMIOLOGY: PRINCIPLES AND CLINICAL APPLICATIONS, has been prepared. In cooperation with the World Health Organization and the World Federation of Neurology Research Group on Neuroepidemiology, a formal course was conducted in Hamburg, West Germany. Another course will be held in Beijing, The People's Republic of China.  A set of video tapes have been produced for training interviewers in the methodology of interviewing for case-control studies. This has been done in both Italian and in English.		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02370-08 NEB
PERIOD COVERED <b>October 1, 1985 through September 30, 1986</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>*Racial and Geographic Differences in Occurrence of Neurologic Disease</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between;"> <span><b>Bruce S. Schoenberg, M.D., Dr.P.H. Chief,</b></span> <span><b>NEB, IRP, NINCDS</b></span> </div>		
COOPERATING UNITS (if any) <b>OBFS, OD, NINCDS: A. Haerer, M.D., Univ. of Mississippi; U.S. Bureau of the Census; C.L. Bolis, M.D., (WHO); B.O. Osuntokun, M.D. (Nigeria); F. Garcia-Pedroza, M.D., (Mexico); Wang Chung-cheng, M.D. (People's Republic of China); N. Bharucha, M.D. (India); M.C. Gutierrez del Olmo, M.D.;**</b>		
LAB/BRANCH <b>Neuroepidemiology Branch, Intramural Research Program</b>		
SECTION		
INSTITUTE AND LOCATION <b>NINCDS, NIH, Bethesda, Maryland 20892</b>		
TOTAL MAN-YEARS  <div style="text-align: center;"><b>4.2</b></div>	PROFESSIONAL:  <div style="text-align: center;"><b>4.2</b></div>	OTHER
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input checked="" type="checkbox"/> (a1) Minors  <input checked="" type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The purpose of this study is to accurately document possible racial differentials in the prevalence of major neurologic disorders by surveying an entire county, with a biracial population of approximately 25,000. The disorders investigated include cerebral palsy, dementia, psychomotor delay, epilepsy, Parkinson's disease, essential tremor, and cerebrovascular disease.</p> <p>In addition, research protocols for neuroepidemiologic studies in developing countries have been prepared for Ecuador, Mexico, Nigeria, Peru, the People's Republic of China, Spain, Venezuela, and India. Pilot investigations have been successfully carried out in Ecuador, Mexico, Nigeria, Peru, the People's Republic of China, and India.</p> <p><b>*[Former title: Racial Differentials in the Prevalence of Major Neurologic Disorders and Surveys in Developing Countries].</b></p>		
** Continued:  <div style="display: flex; justify-content: space-between;"> <div> <b>A. Portera-Sanchez, M.D.,</b>  <b>J. Cabrera, M.D.</b>  <b>P. Ponce, M.D.</b>  <b>M. Cruz, M.D.</b> </div> <div> <b>(Spain)</b>  <b>(Peru)</b>  <b>(Venezuela)</b>  <b>(Ecuador)</b> </div> </div>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02423-07 NEB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Data Resources for Neuroepidemiology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between;"> <div style="width: 60%;">           Bruce S. Schoenberg, M.D., Dr.P.H. Chief,            Lawrence Lavine, M.D. Medical Officer         </div> <div style="width: 35%;">           NEB, IRP, NINCDS            NEB, IRP, NINCDS         </div> </div>		
COOPERATING UNITS (if any) F. Clifford Rose, M.B., FRCP: B. Benjamin, Ph.D., S. Haberman, M.A., F.I.A., and R. Capildeo, M.B., B.S., Charing Cross Neuroepidemiology Unit, London, England; W. Sibley, M.D., Univ. of Arizona, Tucson, AZ; E. Kahahah, M.D., Neurology Unit, Ashkelon, Israel; *		
LAB/BRANCH Neuroepidemiology Branch, Intramural Research Program		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unraduced type. Do not exceed the space provided.)  To develop 1) a registry of hospitalized patients with neurologic disease in a well-defined population of 3.5 million people, 2) resources for case-control studies of neurologic diseases using uniform methods of data collection, 3) to develop a registry of neurologic disease in the well-defined population of the United States military, and 4) a registry of selected neurologic diseases in a well-defined population of 0.5 million people in a county in Hungary.		
----- * Continued  <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;">           Y. Leibowitz            G. Plaffy, M.D.,            G. Gunderson, Col., M.C.(Army)             J. Hallenbeck, CAPT., M.C.,(Navy)         </div> <div style="width: 40%;">           Neuroepidemiology Unit            Neurology, Univ. of Pecs            Dept. of Neurology             Dept. of Neurology         </div> <div style="width: 30%;">           Jerusalem, Israel            Pecs, Hungary            Walter Reed Army Med.                              Center             Bethesda Naval Hospital         </div> </div>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02424-07 NEB
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Standardized Nomenclature and Coding of Neurologic Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  Bruce S. Schoenberg, M.D., Dr.P.H. Chief, <span style="float: right;">NEB, IRP, NINCDS</span>		
COOPERATING UNITS (if any) L. Kurland, M.D., Mayo Clinic, Rochester, MN; J.F. Kurtzke, M.D., Georgetown Univ., Washington, D.C.; F. Clifford Rose, M.B., FRCP, B. Benjamin, Ph.D., S. Haberman, M.A., FIA, and R. Capildeo, M.B., B.S., Charing Cross Neuroepidemiology Unit, London, England; *		
LAB/BRANCH Neuroepidemiology Branch, Intramural Research Program		
SECTION		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  To develop an internationally acceptable standard of nomenclature, classification, and coding of neurologic disorders.		
----- <div style="display: flex; justify-content: space-between;"> <div>           * Continued:            L. Schut, M.D.,            K. Kondo, M.D.         </div> <div style="text-align: right;">           Minneapolis, MN            Tokyo, Japan         </div> </div>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02715-01 NEB

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epilepsy Neuroepidemiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Bruce S. Schoenberg, M.D., Dr.P.H.	Chief,	NEB, IRP, NINCDS
Lawrence Lavine, M.D.	Medical Officer,	NEB, IRP, NINCDS
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COOPERATING UNITS (if any) D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland; J.F. Mellinger, M.S., M.R. Gomez, M.D., L.T. Kurland, M.D., Dr.P.H., and R.V. Groover, M.D., Dept. of Neurology, Mayo Clinic; L.L. Salkowicz, P. Gunderson, Ph.D., Minnesota Dept. of Health; M. Cruz, M.D., Mayo Clinic, MN; \*

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

1.6

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The record-linkage system available for residents of Rochester, Minnesota has been used to identify all possible cases of complex partial, absence, and tonic-clonic seizures diagnosed between the years 1935 and 1979 who were born in the community. Case-control studies were conducted to identify risk factors associated with these seizure types. Potential risk factors studied included mother's characteristics, complications of pregnancy, medical and surgical events during pregnancy, factors of delivery, medical or surgical events at delivery, characteristics of the newborn, and adverse medical events occurring after birth. The relationship between febrile seizures and subsequent risk of epilepsy was specifically addressed.

A study of the relationship of duration of untreated epilepsy to the ease or difficulty of its subsequent treatment is being carried out in several countries. The objective of the study is to determine how critical early diagnosis and early treatment are to the patient in terms of subsequent control of epilepsy.

\* Continued:

Francis Baker, M.D.  
P. Ponce, M.D.

Neurological Disease  
Dept.,  
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Bethesda, Maryland  
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# ANNUAL REPORT

October 1, 1985 through September 30, 1986

National Institute of Neurological and Communicative Disorders and Stroke

## Neuroimmunology Branch

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Annual Report  
October 1, 1985 to September 30, 1986  
Neuroimmunology Branch  
National Institute of Neurological and  
Communicative Disorders and Stroke  
Dale E. McFarlin, M.D., Chief

The Neuroimmunology Branch (NIB) performs both clinical and fundamental investigations which are interrelated and complementary. Emphasis is placed on genetic contributions to immune regulation and on investigation of antigen-specific immune reactivity. A variety of contemporary immunological strategies are used in these studies and over the past year there has been increasing application of molecular biology in NIB research.

NIB clinical research continues to focus on multiple sclerosis (MS). In the past, a variety of immunological findings have been described in this disease, but an antigen-specific abnormality has not been consistently detected. A major finding reported by the Cellular Immunology Section during the past year was that patients with MS have impaired capacity to generate cytotoxic T lymphocytes (CTL) against measles virus. The data indicate that this is specific for MS because the generation of measles specific CTL has been normal in a number of other diseases. Further, the abnormality in MS patients to date, is specific for measles virus because the generation of CTL against influenza and mumps viruses are normal. Recent studies indicate that the reduction in measles-specific CTL in patients with MS is due to a relatively low number of precursor cells with the capacity to differentiate into this effector cell population. It is, however, noteworthy that previous research in the NIB has shown that measles-specific CTL are primarily  $T4^+$  lymphocytes that react with antigen in association with class II molecules of the major histocompatibility complex (MHC) while CTL against influenza virus are predominantly  $T8^+$  and react with antigen in association with class I MHC molecules. Additional investigations are currently in progress to determine the implications of the abnormal findings in MS. Two possibilities are being considered: the first is that the abnormality is specific for measles virus which would implicate this agent in the disease process; the second is that immunological abnormality is limited to a subset of  $T4^+$  lymphocytes with cytotoxic function.

The role of possible genetic influences on the pathogenesis of MS continues to be addressed in twins. A prominent finding in these long-term studies is a much higher concordance of the disease in monozygotic (MZ) than dizygotic (DZ) twins which favors a significant genetic contribution to the pathogenesis. Although emphasis has been placed on genes coding for MHC molecules which are known to have immunoregulatory function, significant difference in the HLA backgrounds between affected and normal DZ twins has not been found. Further, the higher frequency of concordance in MZ twins (over 50%) in our study, as compared to HLA identical DZ twins suggests that the disease is related to more than one gene. This could include genes encoding

for the T cell receptors (Ti) and studies of this possibility are beginning in the NIB.

In the past, abnormalities of cerebrospinal fluid (CSF) immunoglobulins have been detected in many clinically normal twins of patients with MS. The IgG profiles have been analyzed in detail by two-dimensional electrophoresis followed by silver staining by Drs. M. Harrington and Carl Merrill in the National Institute of Mental Health (NIMH). It is of considerable interest that the CSF immunoglobulin composition of individual MZ twin sets tend to be more homogeneous than expected on a random basis. This suggests that immunoglobulin production is nonrandom and specific, at least in part. The CSF findings along with MRI abnormalities in clinically unaffected twins of individuals with MS supports the possibility of subclinical disease.

Therapeutic trials in MS have been an important component of the NIB clinical research. Over the past year the open trial of Poly ICLC in chronic progressive MS has been completed. This was performed in collaboration with Dr. Andre Salazar from the neurology service at Walter Reed Army Medical Center and Dr. H. Levy in National Institute of Allergy and Infectious Diseases (NIAID). Poly ICLC has immunoregulatory properties and is a potent inducer of alpha interferon. Considerable effort was devoted to developing optimal methods for administering the agent and managing side effects, principally fever associated with the treatment. From the study it was concluded that: (1) Poly ICLC can be administered safely to MS patients in a research setting even though many develop significant side effects; (2) most individuals produce large amounts of alpha interferon and steroids; and (3) there was a suggestion that some of the patients either improved or stabilized during the treatment; documentation of this would require a more extensive randomized trial. Cessation of Poly ICLC treatment has not been associated with exacerbations.

Over the past year the quota of patients needed for the randomized double-blind trial of cyclosporine A in actively progressive MS was reached and this protocol is now closed. After detailed clinical assessments, immunological evaluations, and ancillary tests including evoked response studies, as well as MRI which may reflect subclinical changes, patients were randomized into groups which are receiving either cyclosporine A or placebo. The patients are followed at monthly intervals on an outpatient basis and admitted on occasions for repeat immunological evaluations and pharmacokinetic studies. Under the existing protocol each patient will be treated for two years. The treatment period is scheduled to end in 1987 and some indication of efficacy should be available during 1988. In addition to determining information on the effect of cyclosporine A in MS, this population of well-characterized patients is providing valuable baseline information on immunological function, genetic background, and the correlation between ancillary studies useful in the diagnosis of MS.

A significant portion of NIB research is directed at the investigation of

molecular mechanisms responsible for T cell regulation and activation. In these studies the function of molecules on the T cell surface including Ti, T3, T4, T8, T11, and LFA-1 are being examined using a large panel of alloreactive CTL clones. These CTL clones react with the same alloantigen but vary considerably in avidity and in susceptibility to blocking by anti-T3 antibodies. The Ti and T3 molecules are thought to be in close association on the T cell surface. The possibility was considered that the relationship between these two molecules may vary and relate to differences in the susceptibility of individual clones to inhibition by anti-T3 antibodies. When clones that were susceptible to treatment with anti-T3 were compared to other clones that were highly resistant to this treatment, differences were not encountered in the level of surface expression of the T3 complex, the expression of Ti and the capacity of Ti molecules to comodulate with T3. These observations led to the conclusion that interaction between Ti and T3 is relatively constant in the clones that are differentially susceptible to anti-T3 inhibition but have different avidities for their specific targets. In order to examine the Ti molecules in this panel of clones in more detail, a series of molecular genetic experiments designed to examine rearrangements of genes encoding for the Ti beta chains and V beta expression have been initiated.

The fundamental studies of T lymphocyte function have important implications for the cell-mediated immune response (CMI) to various viruses and autoantigens. The target of measles-specific immune cells is being addressed in normals, patients with MS, and patients with other neurological diseases, particularly subacute sclerosing panencephalitis. Measles virus consists of five structural polypeptides and recently developed techniques using monoclonal antibodies and affinity chromatography have led to the successful purification of sufficient quantities of these polypeptides for specificity studies. In the past it was demonstrated that immune cells from individuals with high CMI to measles virus proliferate to all polypeptides. Over the past year, data have been obtained which indicate that a major component of the measles CTL response in the blood is directed at internal virus components such as the nucleocapsid protein. The explanation for these provocative observations is not known, but it is noteworthy that similar findings have been obtained by other investigators studying influenza viruses. Immunolabelling studies have routinely been unsuccessful in demonstrating measles virus nucleocapsid protein on the surface of infected cells. Consequently, it has been postulated that the CTL react with epitopes different from those recognized by antibody and may be small peptide fragments cleaved from the native protein.

Most of the studies of measles CMI have been performed with the Edmonston strain of virus; however, in many infections of humans and animals persistent infection occurs. Consequently, research is currently being conducted on the hamster neurotropic (HNT) strain of measles virus which is neurovirulent and produces persistent infections in experimental animals. During the past year, a series of investigations on the HNT polypeptides were completed in

collaboration with Dr. William Bellini in the Laboratory of Molecular Genetics. Using a panel of monoclonal antibodies a number of differences between the polypeptides of HNT and Edmonston strains were identified. These include: (1) differences in the HA protein which in the HNT strain is slightly smaller and lacks certain epitopes detected by monoclonal antibodies; (2) the phosphoprotein (P) is infrequently synthesized in cells persistently infected with HNT and a smaller protein antigenically related to the phosphoprotein was identified; this may represent the X protein reported by other workers; (3) the nucleocapsid (N) protein is synthesized in HNT but tends to break down; (4) and the matrix (M) protein was not detected.

The absence of the M protein and the lack of authentic size P protein prompted an evaluation of the mRNAs coding for these proteins from cells persistently infected with HNT. Surprisingly, the mRNAs for the M and P proteins were readily identified by Northern analysis using Edmonston-derived cDNA clones. The mRNA encoding for the N protein was also detected. The mRNAs from Edmonston and HNT were analyzed in parallel in an in vitro translation assay. HNT mRNA programmed the synthesis of only the N protein. Collectively, the observations indicate that a variety of genetic and biochemical differences are seen with persistent measles virus infection. These differences are likely to be associated with biological aspects of infection and the immune response to viruses which have important implications for the investigation of such phenomenon in human diseases.

The fine specificity of the human cellular response to measles virus is being addressed with T cell lines and clones. A large panel of measles-specific T cell clones has been produced. Some of these clones proliferate; others have CTL activity, and some both proliferate and have CTL activity. Both the antigen specificity of these clones and their functional capacity are being assessed in in vitro assays.

To date, phenotypic studies have shown that all measles virus-specific T cell clones are T4<sup>+</sup>. As predicted by contemporary immunological dogma, these clones are restricted by class II MHC molecules. It was previously shown that measles-specific CTL derived from a DR2<sup>+</sup>, Dw2<sup>+</sup> patient with MS could lyse some, but not all DR2<sup>+</sup> virus-infected targets. On the basis of mixed lymphocyte reactivity, DR2 is subdivided into five D subtypes; all of these can be infected with measles virus but only two (Dw2 and Dw12) function as effective targets for the CTL from the DR2<sup>+</sup>, Dw2<sup>+</sup> patient. The molecular composition of the class II MHC molecules of the two susceptible D subtypes contained an additional beta chain which was postulated to be the restriction molecule for measles CTL. In order to determine if there was a genetic explanation for these observations which was common to other DR2, Dw2 positive individuals including MS patients, a molecular study of the class II MHC genes in the five D subtypes of DR2 has been initiated in collaboration with Drs. Jack Strominger and Rosa Sorrentino in the Department of Molecular Biology and Biochemistry at Harvard University. Using a probe for the DR2 beta 1 exon, a specific restriction fragment length polymorphism pattern was



identified when one restriction enzyme (Ava II) was used. Extension of this strategy to five MS patients previously known to be homozygous at the DR2 locus has shown an identical pattern. It is noteworthy that the use of a different cDNA probe for DQ beta identified differences at the genetic level which indicate that some of these patients were not homozygotes.

The molecular basis for T cell recognition of Class I MHC molecules is also being investigated. These experiments employ cloned HLA-A genes transfected into murine cells which are used for targets of alloreactive CTL. The results demonstrate that the only gene product required for recognition by alloreactive class I MHC specific CTL is the heavy chain of the class I HLA molecule. The function of other molecules on the surface of CTL are also being examined in experiments using the transfected murine cells as targets.

Immune recognition in the CNS and the mechanisms responsible for the entry of immune cells into the CNS are being studied in murine experimental allergic encephalomyelitis (EAE). The NIB, in collaboration with Dr. Maria Spatz in the Laboratory of Neuropathology, previously obtained data which indicate that CNS endothelial cells (EC) can be induced to express class II MHC molecules and acquire the capacity to function as antigen-presenting cells. These experiments have been extended to examine the expression of Class II MHC (Ia) molecules in mice with EAE produced by passive transfer of immune cells. The findings demonstrate that approximately 25 per cent of EC freshly isolated from mice with EAE express class II MHC molecules. These EC have the capacity to present antigen to immune lymph node cells. It is of considerable interest that studies of kinetics of Ia expression on EC in tissue culture indicate that surface expression of Ia is a relatively short-lived process with a half-life of approximately 24 hours. Parallel experiments showed that the presence of gamma interferon in the tissue culture medium alters this process and leads to Ia expression for a longer period of time and on a greater number of cells; however, even under optimal conditions Ia molecules were expressed on only 70 per cent of the cells.

A variety of immunological stimuli can activate immune cells and lead to Ia expression on EC; this apparently occurs through the release of gamma interferon which acts on the EC. This would have the capacity to upregulate the immune response in vivo. Conversely, other experiments are providing data that EC are potent producers of prostaglandins which downregulate immune reactivity. Collectively, the studies suggest that EC which form the blood-brain barrier, produce and respond to soluble mediators from immune cells and consequently, occupy a critical regulatory influence on cellular immunity in the CNS.

Two years ago a Neuropharmacology Section was established in the NIB with the goal of studying interaction among the immune, endocrine, and nervous systems. Over the past year, this group has initiated two new research projects. The biochemical evaluation of adrenergic function as well as MPTP toxicity and animal models of Parkinson's Disease are being investigated by

the group.

Recent studies have indicated that neurohormones may play important roles in regulating different kinds of immune functions. The capacity of beta-endorphin (b-end) to regulate levels of cytolytic activity and interferon production by human natural killer (NK) cells has been examined. Preincubation of NK cells with  $10^{-7}$  -  $10^{-10}$  M b-end for 2-18 hours produced significant augmentation of NK cytolytic activity and gamma interferon production induced by PHA or poly I:C; these effects of b-end could be specifically blocked by naloxone. These findings lend support to the concepts of regulation of the immune response by neurohormones and the existence of a functional relationship between the nervous and immune systems.

Conversely, recent studies with Drs. B. Roy and D. Murphy in the NIMH have shown that a subgroup of patients with affective disorders have antibodies that react with b-end. These findings have stimulated consideration of the possibility that immune networks consisting of anti-idiotypes directed at anti-peptides operate in parallel to the traditional neuroendocrine systems.

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02202-11 NI

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Studies in Patients with Multiple Sclerosis and other CNS Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dale E. McFarlin, M.D.	Chief	NI	IRP	NINCDS
Others: Henry F. McFarland, M.D.	Deputy Chief	NI	IRP	NINCDS
Andrew Goodman, M.D.	Medical Staff Fellow	NI	IRP	NINCDS
Michael Harrington, M.D.	Visiting Fellow	LGCB	IRP	NIMH
Carl Merril, M.D.	Chief	LGCB	IRP	NIMH
Steven Jacobson, Ph.D.	Sr. Staff Fellow	NI	IRP	NINCDS
Lauren Krupp, M.D.	Medical Staff Fellow	NI	IRP	NINCDS

\*Continued

## COOPERATING UNITS (if any)

LGCB, NIMH

Dept. of Biochemistry and Molecular Genetics, Harvard, Boston

Dept. of Medicine, Johns Hopkins University

## LAB/BRANCH

Neuroimmunology

## SECTION

Office of the Chief

## INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

12.0

## PROFESSIONAL:

8.0

## OTHER:

4.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

Investigation of patients with Neurological Dysfunction. The general aim of this project is to obtain a more precise understanding of the multiple factors possibly related, either singly or in combination, to the pathogenesis of a number of neurological disorders including multiple sclerosis, Sjögrens syndrome, myasthenia gravis, polyneuropathy and other neuromuscular diseases. Focus is currently placed on multiple sclerosis, and studies in this disease consist of a detailed evaluation of the immunological function and immunogenetic background as well as therapeutic trials. Magnetic resonance imaging is being used to assess the extent and magnitude of lesions in the white matter. These studies are performed in patients with sporadic disease, patients with a family history of demyelinating disease as well as identical and nonidentical twins who are either concordant or discordant for the disease. Immunological studies include assessment of cellular immune reactivity to a number of viruses and the analysis of cell surface molecules on leukocytes in the blood and cerebrospinal fluid. Immunoglobulin production and specificity are being evaluated by highly sensitive techniques. Trials of experimental therapeutic approaches are conducted in carefully selected patients with multiple sclerosis. A phase I trial of Poly ICLC, an interferon inducer, has been completed. In another phase I trial blood lymphocytes are transferred from a normal person to an identical twin with multiple sclerosis. A phase III cooperative trial of cyclosporine A in chronic progressive multiple sclerosis is currently in progress. Approximately fifty patients have been randomized into groups that are treated with either cyclosporine A or placebo. During therapeutic trials both the clinical status and immune function are monitored.

\*Suhayl Dhib-Jalbut, M.D.  
Jack Strominger, Ph.D.  
Elaine Alexander, Ph.D.

Visiting Associate	NI	IRP	NINCDS
Professor	Harvard University		
Assistant Professor	Johns Hopkins University		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02203-11 NI
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Immune Response Against Membrane Antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Dale E. McFarlin, M.D. Others: Henry F. McFarland, M.D. W. J. Bellini, Ph.D. John Rose, M.D. S. Dhib-Jalbut, M.D. J. Sever, M.D.	Chief Deputy Chief Special Expert Medical Staff Fellow Visiting Associate Chief	NI NI LMG NI NI ID IRP IRP IRP IRP IRP NINCDS NINCDS NINCDS NINCDS NINCDS NINCDS
COOPERATING UNITS (if any) LMG, NINCDS ID, NINCDS		
LAB/BRANCH Neuroimmunology		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The major goal of this project is to characterize measles virus antigens and other components expressed on the surface of infected cells which are the targets of the immune response. <u>Monoclonal antibodies</u> against the five structural proteins of measles virus have been produced and are used to characterize and purify these viral components. Three components, the <u>hemagglutinin (HA)</u>, the <u>fusion (F)</u>, and the <u>matrix (M)</u> proteins are incorporated into the membrane. The <u>nucleocapsid associated protein (N)</u> and the <u>phosphoprotein (P)</u> are internal components. Each of the virus proteins has unique function and immunological properties. The cellular immune responses to epitopes on each of the five proteins are being assessed by lymphocyte proliferation and cytotoxicity.</p> <p>Measles virus infection can be complicated by persistent infection of the nervous system and although most of the research is being conducted with the Edmonston strain, the <u>hamster neurotropic strain (HNT)</u> which is neurovirulent and characteristically produces CNS infections in experimental animals is also being studied. The biological, immunological, and molecular properties of Edmonston and HNT strains are being compared. Relationships between human MHC molecules and the measles antigens are being examined.</p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02204-11 NI																																			
PERIOD COVERED October 1, 1985 through September 30, 1986																																					
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Immunologic Mechanisms Operative in Experimental Autoimmune Diseases of the Nervous System</b>																																					
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 35%;">PI: Dale E. McFarlin, M.D.</td> <td style="width: 35%;">Chief</td> <td style="width: 30%;">NI</td> <td style="width: 10%;">IRP</td> <td style="width: 10%;">NINCDS</td> </tr> <tr> <td>Others: Richard McCarron, Ph.D.</td> <td>Sr. Staff Fellow</td> <td>NI</td> <td>IRP</td> <td>NINCDS</td> </tr> <tr> <td>Anne Cross, M.D.</td> <td>Medical Staff Fellow</td> <td>NI</td> <td>IRP</td> <td>NINCDS</td> </tr> <tr> <td>Ute Traugott, M.D.</td> <td>Assistant Professor</td> <td>Albert Einstein U.</td> <td></td> <td></td> </tr> <tr> <td>Cedric Raine, Ph.D.</td> <td>Professor</td> <td>Albert Einstein U.</td> <td></td> <td></td> </tr> <tr> <td>Maria Spatz, M.D.</td> <td>Section Head</td> <td>LNNS</td> <td>IRP</td> <td>NINCDS</td> </tr> <tr> <td>Oliver Kempfski, M.D.</td> <td>Visiting Fellow</td> <td>LNNS</td> <td>IRP</td> <td>NINCDS</td> </tr> </table>			PI: Dale E. McFarlin, M.D.	Chief	NI	IRP	NINCDS	Others: Richard McCarron, Ph.D.	Sr. Staff Fellow	NI	IRP	NINCDS	Anne Cross, M.D.	Medical Staff Fellow	NI	IRP	NINCDS	Ute Traugott, M.D.	Assistant Professor	Albert Einstein U.			Cedric Raine, Ph.D.	Professor	Albert Einstein U.			Maria Spatz, M.D.	Section Head	LNNS	IRP	NINCDS	Oliver Kempfski, M.D.	Visiting Fellow	LNNS	IRP	NINCDS
PI: Dale E. McFarlin, M.D.	Chief	NI	IRP	NINCDS																																	
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Maria Spatz, M.D.	Section Head	LNNS	IRP	NINCDS																																	
Oliver Kempfski, M.D.	Visiting Fellow	LNNS	IRP	NINCDS																																	
COOPERATING UNITS (if any) Departments of Pathology (Neuropathology) and Neuroscience, Albert Einstein College of Medicine, New York, N.Y.																																					
LAB/BRANCH Neuroimmunology																																					
SECTION Neurological Diseases Section																																					
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892																																					
TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 2.0	OTHER: 1.5																																			
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																																					
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The mechanisms responsible for the production of experimental allergic encephalomyelitis (EAE), a model of autoimmune disease which is manifested by <u>demyelination</u>, are being examined. The studies are being conducted in mice because this species is ideally suited for the analysis of immunologic and genetic factors related to disease. Three forms of the murine disease have been produced: 1) Acute EAE, 2) <u>Chronic Relapsing EAE</u> and 3) <u>Adoptively Transferred EAE</u>. Current research is focused on the adoptively transferred model. The transfer of lymphocytes sensitized against myelin basic protein leads to neurological dysfunction characterized pathologically by <u>inflammation and primary demyelination</u>. Many mice recover and develop chronic relapsing disease. The mechanisms responsible for both the initial and the chronic disease are not known, but an early event is the migration of immune cells across the <u>blood-brain barrier</u> into the central nervous system (CNS). The subpopulation of T-lymphocytes which is responsible for the disease are <math>Lyl^+ 2^-</math> and react with antigen in association with class II MHC (Ia) molecules. Immunohistochemical examination of CNS endothelial cells in tissue section and the cytofluorographic studies of whole cells freshly isolated from the CNS indicate class II MHC molecules are not expressed on normal endothelial cells but appear during the development of EAE. Immune lymph node cells with the capacity to transfer EAE produce gamma interferon which can induce the expression of Ia molecules on endothelial cells. These observations have led to the hypothesis that interaction between immune cells and the capillary endothelium leads to alterations of the blood-brain barrier.</p>																																					

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02205-11 NI
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Interaction Between Viruses and the Host Immune System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Henry F. McFarland, M.D. Others: Dale E. McFarlin, M.D. Steven Jacobson, Ph.D. William E. Biddison, Ph.D. John W. Rose, M.D. John R. Richert, M.D. Andrew Goodman, M.D. Lauren Krupp, M.D.	Deputy Chief Chief Senior Staff Fellow Senior Investigator Medical Staff Fellow Assistant Professor Medical Staff Fellow Medical Staff Fellow	NI, IRP, NINCDS NI, IRP, NINCDS NI, IRP, NINCDS NI, IRP, NINCDS NI, IRP, NINCDS Georgetown University NI, IRP, NINCDS NI, IRP, NINCDS
COOPERATING UNITS (if any) Department of Neurology, Georgetown University		
LAB/BRANCH Neuroimmunology		
SECTION Cellular Immunology Section		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS. 4.5	PROFESSIONAL: 4.0	OTHER. 0.5
CHECK APPROPRIATE BOXES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided ) <p>             The purpose of this project is to evaluate the immune response to viruses in healthy individuals and to identify abnormalities in these responses which may be related to the <u>disease mechanisms</u>. These studies focus on functional analysis of the cellular immune response to viruses which commonly affect humans and in particular measles virus. The mechanisms involved in the regulation of these responses are being examined. An additional goal of these studies is to evaluate the influence of genetic makeup on both induction and effector phases of the immune response to viruses. Assays for <u>cytotoxic T-cells</u>, <u>amplifier T-cells</u> and <u>suppressor T-cells</u> directed at viruses are being developed. Virus antigens recognized by these cell populations and the influence of antigen presentation on the generation of these responses are being examined. Finally, these investigations apply findings obtained in healthy individuals to the evaluation of patients with the diseases of the nervous system, particularly multiple sclerosis which may involve immunological or infectious mechanisms. Longitudinal studies of both normal individuals and patients will be conducted.           </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02603-03 NI
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Molecular Mechanisms of Lymphoid Cell-Cell Interactions</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: William E. Biddison, Ph.D. Others: Mary L. Jelachich, Ph.D. Elli Leontsini, M.D. Steven Beall, M.D. Elliot Cowan, Ph.D. John Coligan, M.D. David Nelson, M.D.	Senior Investigator Guest Researcher Visiting Fellow Medical Staff Fellow Staff Fellow Section Chief Senior Investigator	NI NI NI NI NI LIG MET B IRP IRP IRP IRP IRP IRP IRP NINCDS NINCDS NINCDS NINCDS NINCDS NIAID NCI
COOPERATING UNITS (if any)  LIG, NIAID MET B, NCI		
LAB/BRANCH Neuroimmunology		
SECTION Cellular Immunology		
INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 6.0	PROFESSIONAL: 3.0	OTHER: 3.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The goal of this project is to define the mechanisms by which cell surface molecules function in T cell recognition of foreign cell surface antigens. Studies of the functional roles of the T cell surface molecules T3, T4, and LFA-1 demonstrated that there was an inverse correlation between the avidity of individual T4<sup>+</sup> CTL clones for their specific targets and their susceptibility to inhibition by anti-T3 and anti-T4, but not anti-LFA-1, monoclonal antibodies. The quantity and interaction of cell surface antigen-specific T cell receptor (Ti) molecules and the T3 complex were examined on those CTL clones that are of the highest avidity and most resistant to blocking by anti-T3 antibodies to examine the hypothesis that the Ti molecules on these clones were not strongly physically associated with the T3 complex. No significant differences were found between highly resistant and susceptible clones for: 1) the levels of cell surface expression of the T3 complex and Ti; 2) the ability to modulate T3 cell surface molecules; and 3) the capacity of the Ti molecules to co-modulate with the T3 complex. The most likely explanation for the observed heterogeneity in susceptibility to blocking by anti-T3 antibodies is, therefore, thought to be that individual CTL clones possess Ti molecules with differential avidity for specific targets.           </p> <p>             The dissection of the molecular basis for T cell recognition of class I HLA antigens has been continued with the use of cloned HLA-A3 genes transfected into mouse cells by DNA-mediated gene transfer. HLA-A3-specific allo-reactive CTL and HLA-A3-restricted influenza virus-specific human CTL have been shown to be able to specifically lyse the appropriate mouse cell transfectants. These results demonstrate that the only human gene product required on target cells for recognition by allo-reactive and HLA-restricted class I CTL is the HLA heavy chain.           </p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 NS 02716-01 NI
<b>PERIOD COVERED</b> October 1, 1985 through September 30, 1986		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) <b>MPTP Toxicity and Animal Models of Parkinson's Disease</b>		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) P.L. Irwin J. Kopin, M.D., Chief, Immunopharmacology Section, NIB, NINCDS Others: Krzysztof S. Bankiewicz, Visiting Fellow, M.D., SNB, NINCDS Virginia Weise, Immunopharmacology Section, NIB, NINCDS Giovanni Corsini, M.D., Visiting Scientist, Immunopharmacology Section, NIB, NINCDS Urmi Patel, Ph.D., Sr. Staff Fellow, Immunopharmacology Section, NIB, NINCDS *		
<b>COOPERATING UNITS</b> (if any) Surgical Neurology Branch, NINCDS Laboratory of Clinical Science, NIMH		
<b>LAB/BRANCH</b> Neuroimmunology Branch		
<b>SECTION</b> Immunopharmacology		
<b>INSTITUTE AND LOCATION</b> NINCDS, NIH, Bethesda, MD 20892		
<b>TOTAL MAN-YEARS:</b> 3.2	<b>PROFESSIONAL:</b> 2.2	<b>OTHER:</b> 1.0
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.) <p>1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin which causes selective destruction of dopaminergic neurones of the nigro-striatal pathway in monkeys, but is less specific in mice requiring higher doses and affecting other areas as well. A hemiparkinsonian model has been developed by infusion of MPTP into the internal carotid artery of monkeys. The behavioral effects (turning in the direction of the lesioned side) provide a useful means for functional evaluation of agonists (which reverse the turning) and of effects of procedures to stimulate regeneration or replace by implantation dopamine-producing cells. The involvement of the dopaminergic neurones and changes in receptor properties as a result of neuronal damage are being examined using biochemical techniques for measurement of dopamine, norepinephrine, and serotonin and their metabolites as well as binding of radio-labelled ligands to receptors and immunohistofluorescence of tyrosine hydroxylase. These confirm unilateral destruction of dopamine innervation of the caudate-putamen in hemiparkinsonian monkeys and increased D<sub>2</sub> receptors in this area in these animals. PET scanning with <sup>18</sup>F-DOPA is being planned to follow the degeneration-regeneration process in monkey brain after MPTP treatment. The mechanisms of toxicity involve metabolism to its pyridium derivative (MPP<sup>+</sup>) which is accumulated in dopaminergic neurones. Toxicity of MPP<sup>+</sup> may involve free radicals and affinity to neuromelanin. In mice, drugs which potentiate (e.g., copper chelators) or alleviate (e.g., reducing agents) MPTP toxicity provide evidence for involvement of superoxide free-radicals in causing the toxic damage to the amine accumulating neurones.</p> <p>-----</p> <p>*Continued:          Tomoyoshi Kondo, M.D., Visiting Fellow, Immunopharmacology Section, NIB, NINCDS          Sergio Schinelli, M.D., Ph.D., Visiting Fellow, Immunopharmacology Section, NIB, NINCDS          Chuang Chiehueh, Ph.D., Special Expert, LCS, NIMH          Sanford Markey, Ph.D., Chief, Section on Analytical Biochemistry, LCS, NIMH          Jan Johannessen, Ph.D., Sr. Staff Fellow, LCS, NIMH          Edward Oldfield, M.D., Acting Chief, SNB, NINCDS          David Jacobowitz, Ph.D., Chief, Section on Histopharmacology, LCS, NIMH</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02717-01 NI

PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Evaluation of Adrenergic Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.L.: Irwin J. Kopin, M.D., Chief, Immunopharmacology Section, NIB, NINCDS  
Others: Graeme Eisenhofer, Ph.D., Visiting Fellow, Immunopharmacology Section, NIB, NINCDS  
Zofia Zukowska-Grojec, M.D., Ph.D., Guest Worker, Immunopharmacology Section, NIB, NINCDS  
David Goldstein, M.D., Ph.D., Hypertension-Endocrine Branch, NHLBI  
\*

COOPERATING UNITS (if any)

Medical Neurology Branch, NINCDS  
Hypertension-Endocrine Branch, NHLBI

LAB/BRANCH

Neuroimmunology Branch

SECTION

Immunopharmacology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.2

PROFESSIONAL:

3.2

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Measurement levels of norepinephrine (NE) and its metabolites in plasma, urine, and cerebrospinal fluid have been developed and are being applied to studies of changes of these metabolites in patients with various disorders of the autonomic nervous system and in rats after administration of drugs which alter synthesis, storage, release, metabolism, or uptake of NE in sympathetic nerves. Consistent with metabolism of NE by monoamine oxidase after neuronal uptake, drugs such as desipramine (or cocaine) which block uptake, enhance apparent release of norepinephrine into the circulation and diminish formation of its deaminated metabolites DHPG and MHPG. Other studies focus on the regulation of release by peptides such as Vasoactive Intestinal Peptide and Atrial Natriuretic Factors. These studies are carried out in pithed rats. The relationship between plasma NE levels and concentrations at the neuroeffector junction are determined by measurements of NE in plasma during NE infusion or NE release from sympathetic neurones in relation to responses. This method, developed in pithed rats is being applied to studies in human forearm. In the course of such studies, venous-arterial differences in L-DOPA have been found and it appears that L-DOPA is formed in the sympathetic neurones and overflows into the blood.

\*Continued:

Markus Haass, M.D., Visiting Fellow, Immunopharmacology Section, NIB, NINCDS  
Ronald Polinsky, M.D., Chief, Section on Clinical Pharmacology, Medical Neurology Branch, NINCDS







ANNUAL REPORT

October 1, 1985 through September 30, 1986

Surgical Neurology Branch

National Institute of Neurological and Communicative Disorders and Stroke

Edward H. Oldfield, M.D., Acting Chief

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## ANNUAL REPORT

October 1, 1985 through September 30, 1986  
Surgical Neurology Branch, IRP  
Edward H. Oldfield, M.D., Acting Chief

National Institutes of Neurological and Communicative Disorders and Stroke

### I. SUMMARY OF STUDIES

The Surgical Neurology Branch (SNB) principal activities are conducted in the following sections: 1) Clinical Neurosurgery Section; 2) Tissue Culture Laboratory; 3) Neuroimmunology Unit; 4) Cellular Biology Unit; 5) Biochemistry Unit; and 6) Electron Microscopy Unit.

The SNB has as its major research function the study of the biology and therapeutic approaches to the problem of malignant tumors of the brain. Its clinical function is to provide the neurosurgical services to its own research protocol patients and to patients seen in consultation in the NIH Clinical Center. The SNB is presently located in Building 10A, 10 and 9. Its staff includes 14 clinical neurosurgeons at various levels of training and experience as well as 1 senior scientist, 4 junior scientists and a support staff of technical and administrative individuals.

In addition to its primary functions of clinical and basic research the SNB provides young neurosurgeons with experience in clinical & laboratory investigation in a combined clinical and neuroscience environment. Of the 30 individuals who have participated in the SNB program as medical staff fellows and senior staff fellows, most have entered or are planning to enter academic roles in neurosurgery.

In the SNB research program, there have been significant advances within the past year. These advances have been at basic research and clinical levels. It has been found that IL-2 (Interleukin 2), a lymphokine, can stimulate autologous lymphocytes from glioma patients to become specifically cytotoxic to the tumor cells of that patient. This laboratory observation has been extrapolated to the clinical area and patients have been treated with these "LAK" cells, administered directly into their tumors.

The use of intraarterial therapy with subsequent drug removal using a dialysis cartridge drug removal has shown both experimental and clinical promise in improving chemotherapy for brain tumors. It has been shown that the drug spirohydantoin, a "dilatant mustard", has specific efficacy in brain tumors without the bone marrow suppression characteristic of other agents. Furthermore, this agent causes a confusional state similar to Alzheimer's disease which is completely reversible with intravenous physostigmine. The potential role of differentiation agents has been suggested by in vitro studies which have shown not only tumor cell growth suppression and morphological differentiation but also a decrease in plasminogen activator secretion.

The research program has been established to work on many of the basic questions in glioma biology. The clinical program provides neurosurgical care to address the problems of glioma patients at any stage of disease.

## A. CLINICAL NEUROSURGERY SECTION

Edward H. Oldfield, M.D., Chief

The clinical activities of the Surgical Neurology Branch are primarily directed to the investigation of the biological behavior and mechanisms of pathophysiology of malignant primary brain tumors, pituitary tumors, acoustic neurinomas, spinal cord AVMs, and the surgical management of epilepsy refractive to medical therapy.

### Malignant Primary Brain Tumors

#### 1. Intraarterial Chemotherapy

One of the basic tenets of anticancer chemotherapy is that increased tumor exposure to drug should result in increased tumor response. One method currently being used to increase drug exposure of malignant brain tumors is by intracarotid infusion. Although intracarotid infusion increases drug delivery, systemic toxicity, not brain toxicity, frequently limits the tolerable dose. A means of extracting the drug from the blood after one passage through the brain, and before the high concentration of drug reaches the general circulation, should allow dose escalation to levels which provide increased tumor response. We have attempted to develop techniques which provide regional vascular isolation of a tumor-bearing region by percutaneous catheterization of the afferent and efferent vessels. This permits the venous drainage of the region to be circulated extracorporeally for drug removal before the high concentration of drug reaches the systemic circulation. The following have been demonstrated.

- a) A pilot study of 4 patients treated with intracarotid BCNU during extracorporeal hemoperfusion of the jugular venous blood demonstrated that the drug exposure of the body could be reduced by 56-87% by channeling the blood extracorporeally at 300 ml/min through a hemoperfusion cartridge for drug extraction. The pharmacokinetic advantage (Ratio of brain exposure to body exposure) compared to intravenous infusion of the same dose was 21-55:1.
- b) Techniques to deliver intracarotid infusions of BCNU distal to the origin of the ophthalmic artery were evaluated and found successful and have now been shown to eliminate the retinal toxicity and visual loss which frequently occur after intracarotid infusion of BCNU into the portion of the carotid artery proximal to the ophthalmic artery.
- c) Drug streaming during intracarotid delivery results in maldistribution



of the infused agent with the potential of delivering very high (toxic) concentrations of drug to some regions of the brain, while other areas received minimal drug. Patients who received intracarotid BCNU were studied with CT scanning, PET scanning, and MRI and the results correlated with histopathology to demonstrate that 1) progressively enlarging cerebral lesions which are often seen on CT and MRI scans following intracarotid chemotherapy may not be tumor recurrence but sites of focal cerebral necrosis; 2) drug streaming is probably the cause of focal encephalomalacia following intracarotid infusion of BCNU.

- d) Drug streaming was studied in rhesus monkeys by comparing the distribution of the deposition of  $^{14}\text{C}$ -iodoantipyrine during two methods of intracarotid infusion. A rapid retrograde infusion eliminated the prominent heterogeneous distribution of drug deposition which occurred during the slow infusion (the slow infusion was at a rate analogous to that which is currently being generally used clinically). This study strongly suggests that current methods of intraarterial drug delivery, to the brain and other sites, are associated with an unpredictable and variable drug distribution and that this maldistribution can be eliminated by techniques, such as a rapid infusion, which eliminate intraarterial streaming.
- e) In experiments recently completed in the DRS by Drs. Shook, Dedrick, Lutz and Doppman, drug streaming was eliminated in an in vitro model of the human carotid arterial system by pulsed intraarterial infusion during diastole. This is now being evaluated in rhesus monkeys with pulsed diastolic injection of labeled  $^{14}\text{C}$ -iodoantipyrine into the internal carotid artery. The pulsed diastolic injection is being performed with a special pump that is linked to the cardiac cycle so that infusate enters the vessel during the diastolic portion of the flow in the parent vessel. Early results using quantitative autoradiography suggest that this technique will allow drug delivery into an arterial vessel with the elimination of drug streaming, and will do so at flow rates that can be used without difficulty clinically. We are now using this system to deliver high dose cisplatin ( $200 \text{ mg/m}^2$ ) into the internal carotid artery of humans.
- f) A dye dilution study of intracarotid infusion of indocyanine green in rhesus monkeys demonstrated that the recovery of substances from the jugular blood after intracarotid infusion is linearly related to the rate of aspiration of the blood from the jugular blood. We have recently demonstrated a similar relationship using indocyanine green during intracarotid drug infusion in humans. This suggests that the amount of an injected drug which can be removed from blood before the blood is returned to the body is dependent on the rate at which blood is withdrawn from the jugular vein. With an effective extracorporeal device for extracting drug, it may be possible to remove as much as 95% of an injected dose before the drug reaches the general circulation.
- g) A preliminary study, performed in vitro, demonstrated that about 90% of the cisplatin in whole blood circulating at  $300 \text{ ml/min}$  could be removed

by hemodialysis using 2 hemodialysis cartridges in series. We then treated 4 patients with cisplatin by intracarotid infusion of a dose that is widely used intravenously. Extracorporeal circulation of the jugular blood for drug removal during intracarotid infusion resulted in tumor exposures 5-15 fold greater than the exposure of the remainder for the body. We have now performed 3 treatments in humans using a very high dose of cisplatin (200 mg/m<sup>2</sup>) combined with drug removal by extracorporeal hemodialysis. The results of the systemic drug levels suggest that the body was exposed to less than 1/2 of the exposure expected if the drug-removal technique had not been used. It is too early to evaluate tumor response, but there has been no apparent brain injury from the high-dose intracarotid infusion.

The above studies were performed in collaboration with Dr. Robert Dedrick of the Bioengineering and Instrumentation Branch of the Division of Research Services and Dr. John Doppman of the Diagnostic Radiology Department, The Clinical Center.

## II. Elaboration of a Factor by Malignant Gliomas Which Increases the Permeability of Normal Blood Vessels In Vivo

One of the pathophysiological mechanisms of the production of an intracranial mass effect by primary and secondary malignant tumors is by the tumor eliciting cerebral edema in the surrounding normal tissue. We have demonstrated that media from malignant gliomas in monolayer cultures secrete a substance which, when injected intradermally into guinea pigs, increases the accumulation of a circulating radioisotope (I<sup>125</sup>RISA) and a marker dye (methylene blue) at the site of injection compared to media from fibroblasts, benign brain tumors, normal saline and tissue culture media. The production of the increased vascular permeability factor in the media from malignant gliomas can be abrogated by incubation of the tumor cells with dexamethasone and by inhibition of protein production by cyclophosphamide. Preliminary work of isolation of the factor suggests that it is a 35,000-45,000 molecular weight protein.

## III. PET Scanning While Using Barbiturate Anesthesia to Accentuate the Difference in Glucose Metabolism of Malignant Gliomas and Normal Brain

This project involves FDG-PET scanning of patients who have brain tumors before and during deep barbiturate general anesthesia. The results indicate that a profound reduction of cerebral glycolytic activity can be achieved with a level of anesthesia which produces burst-suppression EEG activity. Gliomas, however, have only a minimal change in glycolytic rate under the barbiturate anesthesia. The results also indicate that lower grade lesions, which are not visible on PET scans performed with the patient awake, become visible as background synaptic activity is suppressed with barbiturates. The true extent of growth into the surrounding tissue by higher grade lesions can be better appreciated when background activity is reduced under the barbiturate anesthesia. This work provides evidence that barbiturates may allow a

"reverse contrast enhancement" of lesions with decreased neuronal activity. This phenomenon may provide basis for development of specific antitumor therapy. This technique also may be valuable in studying other pathological processes such as degenerative diseases, epilepsy, movement disorders, cerebral infarction and head injury.

#### IV. Positron Imaging of Human Tumor Response to Interstitial Radiation- Donald C. Wright, M.D.

A group of patients have been examined by gallium-EDTA and  $^{18}\text{F}$ -2-deoxyglucose (FDG) PET scans. The focal and global effects of high dose irradiation on cerebral glucose metabolism and capillary permeability are being assessed. The temporal profile of reactive brain edema (Gallium-EDTA) to radiation, and the degree of radiation necrosis are the primary goals of this investigation. We are also examining the effects of steroids on the transport kinetics in tumor and peritumoral regions of humans with malignant gliomas who receive intratumoral implants of radioactive seeds.

#### V. Systemic Chemotherapy - Conrad Kufta, M.D.

##### 1. Spiromustine Phase I Study

The disease specific phase I trial of spiromustine, a drug synthesized to cross the blood brain barrier and function as a tumoricidal agent, was closed in 1986. Twenty-three patients with high grade glioma and one patient with metastatic paraganglioma have been entered into the study and were treated at escalating doses until a maximum tolerated dose (MTD) was identified.

The dose-limiting toxicity of spiromustine was found to be an acute neurologic syndrome manifested chiefly as acute confusion with visual and auditory hallucinations and fixed dilated pupils. This syndrome was identified as an anticholinergic effect by the rapid reversal of its components with parenteral physostigmine. Despite antagonism of the anticholinergic effects of spiromustine with physostigmine, at doses above  $9.9 \text{ mg/m}^2$  a substantial number of patients became stuporous or comatose for short periods after treatment. This is considered unacceptable in patients with increased intracranial pressure. Hence the MTD was defined at  $9.9 \text{ mg/m}^2$ .

Clinical response, determined within the limits of a phase I study, suggests activity. One patient has a complete remission sustained over twenty-eight months and eight additional patients have had partial responses determined by clinical and/or CT scan criteria.

No permanent or late adverse effects have been noted to date on neurologic, neuropsychologic, hematologic or general physical examination. There is virtually no myelosuppression at any dose level, even in heavily pretreated patients, which suggests a potential role for spiromustine in combination chemotherapy protocols if data from phase II trials confirm these early results.

The marked and prolonged central anticholinergic effects of spiromustine were studied in patients by positron emission tomography (PET) using the  $^{18}\text{F}$ -2-deoxyglucose technique. PET studies of cerebral glucose metabolism showed a large global decrease in cerebral glucose utilization of between 35% and 50% of pretreatment values, which involved equally all cortical, hippocampal and basal ganglia regions. Parenteral physostigmine was then given to one of the patients who was re-scanned following a second dose of  $^{18}\text{F}$ -2-deoxyglucose. This demonstrated the return of cerebral metabolism to within 5% of the pretreatment value. This study documents not only the marked central anticholinergic activity of spiromustine and its reversal by anticholinesterase therapy, but also demonstrates how selective disturbance of one transmitter system may result in almost global disruption of cerebral function.

The effects of spiromustine on the cholinergic system in the Sprague-Dawley rat brain were studied. In one study choline uptake, choline acetyltransferase activity, and muscarinic receptor binding were shown to be reduced in synaptosomal preparations from rat frontal cortex, basal ganglia and hippocampal regions after pretreatment with spiromustine. Different brain regions were effected to different degrees, which may represent different drug uptake or differences in affinity of receptor subtypes in these regions.

In another set of experiments physostigmine was shown to reduce the severity of CNS effects (primarily manifested as convulsions) of spiromustine in NIH OM rats. High dosage choline loading was shown in early experiments to have similar protective effect.

## 2. AZQ Trial in Malignant Glioma

The phase II trial of AZQ in patients with malignant glioma was formally closed in 1985. Survivors continue to be followed in the Surgical Neurology Branch Clinic. To date long term survivors (survival greater than two years after initiation of therapy) represent approximately 12% of all patients who were treated.

Follow-up has revealed no evidence of late or progressive toxicity in survivors.

## VI. QUANTITATIVE AUTORADIOGRAPHY WAS USED TO INVESTIGATE SEVERAL ASPECTS OF THE METABOLISM AND THERAPY OF BRAIN TUMOR MODELS IN ANIMALS- Donald C. Wright, M.D.

Since glucose metabolism has been demonstrated to be higher in malignant brain tumors than in the surrounding brain tissue and 2-deoxy-D-glucose metabolism is blocked after initial phosphorylation intracellularly, it may be possible to use 2-deoxy-D-glucose as a tumoricidal agent. The following studies were designed to investigate this possibility.

## Pharmacokinetics

A two part study of the kinetics of tracer amounts (verifying prior studies) and pharmacologic doses of intravenous and intraperitoneal 2-deoxy-D-glucose was performed in normal rats. The study demonstrated differing blood and tissue time courses of tracer vs. pharmacologic doses of 2DG. Toxicologic (LD<sub>50</sub>) data included blood and tissue levels in surviving and dying animals. A related experiment evaluated the effect of repeated pharmacologic doses and various schedules to find a combination of dose and interval which would minimize toxicity.

Following these preliminary studies a series of rats bearing subcutaneous and intracerebral Walker-256 tumors were "treated" with pharmacologic doses of 2DG. The half lives for the drug in tumor were 2.5-3 times that of normal tissue. An examination of the "lumped constant" for 2DG in pathologic tissue was carried out using a quantitative method (autoradiography) and drug schedules were designed for future treatment studies to minimize toxicity.

### 1. 2-deoxy-glucose (2DG) as an Adjunct to Radiation Therapy of Tumors

2DG was effective in killing subcutaneous tumors in rats. Its mechanism of action makes it an attractive treatment for tumors in synergy to optimize treatment of subcutaneous Walker 256 tumors in rats. A pilot series of rats bearing subcutaneous W-256 were given a five day course of (pharmacologic doses) 2DG combined with five different doses of irradiation delivered on days 2-4 of the 2DG "therapy". Additive effects of 2DG + XRT were demonstrated (measured by tumor volumes). Future studies are planned to assess the effects on an intracerebral model.

### 2. Streaming in Normal Rat Microcirculation

A quantitative demonstration of laminar-flow streaming in the rat arterial microcirculation supplements earlier observations showing this phenomenon influences drug distribution in an intra-arterial delivery. Slow, intermediate, and rapid infusions were compared with intravenous infusions using 14-C-iodoantipyrine to determine the degree in which streaming influences solute distribution.

### 3. Intratumoral Drug Delivery in Dogs

A technique for constant/intermittent delivery of solutes of solid tumors is under study in normal and tumor bearing dogs. A totally implantable reservoir allows percutaneous infusions of various solutes. The drug diffusion profiles around catheters placed in normal and neoplastic tissue are being quantitated, as is the washout curve from normal brain, and CSF drug levels resulting from intraparenchymal/intratumoral infusions.

#### 4. Effect of Dexamethasone on Cerebral Edema Following Freeze Injury

A freeze injury model that we had previously developed was used to investigate steroid effects on capillary permeability and extracellular space volumes. <sup>125</sup>IAlbumin was infused following production of a cortical freeze injury in rats. A control/steroid pretreatment group comparison showed a decrease in the volume of the extracellular space in the dexamethasone group.

#### 5. Gallium-EDTA Positron Imaging

Tumor bearing dogs were studied with <sup>68</sup>Gallium-EDTA positron scanning to investigate the integrity of the blood-brain-barrier of this tumor model. Quantitative analysis of influx and efflux constants, and vascular and extracellular spaces was performed. A series of tumor bearing (Avian sarcoma virus induced) dogs were also imaged using dynamic PET scans. The effect of steroids on these physiological parameters is being studied.

#### Pituitary Tumors

#### VII. VENOUS SAMPLING TO ESTABLISH THE DIAGNOSIS AND LOCATION OF HORMONE-SECRETING PITUITARY MICROADENOMAS - Edward H. Oldfield, M.D.

We have now performed bilateral and simultaneous inferior petrosal sinus sampling in 96 patients with Cushing's syndrome. The results are used 1) to confirm in diagnosis of patients preoperatively and, 2) to determine the half of the pituitary gland in which a microadenoma resides. The study has been particularly rewarding and has demonstrated the following: 1) sampling from both inferior petrosal sinuses simultaneously consistently distinguishes patients with Cushing's disease from those with ectopic ACTH syndrome; 2) sampling from a single inferior petrosal sinus, as has previously been general practice, to establish the diagnosis of Cushing's syndrome, may be misleading and could result in an incorrect assumption of the source of excess ACTH secretion in as many as half the patients with Cushing's syndrome; 3) preoperative sampling for ACTH concentrations in the inferior petrosal sinuses determines the site of ACTH-secreting microadenomas within the pituitary gland. Therefore, the surgeon's search for small microadenomas can be focused to one side of the gland, which should be helpful in finding smaller tumors. If no tumor is found, the half of the gland containing the microadenoma can be removed. We have no treatment failures in the 48 previously untreated patients with Cushing's disease who have undergone preoperative sampling. The technique has also been used to locate the site of one prolactin-secreting and one TSH-secreting microadenoma preoperatively. This technique, which we recently introduced here at the NIH, is now being widely employed in the evaluation of patients with Cushing's syndrome.

#### Development of Primate Model of Unilateral Parkinson's Disease

We have developed a new model of Parkinson's disease which allows in vivo

quantification of response to therapeutic maneuvers. Systemic administration of MPTP selectively injures the nigrostriatal pathway in primates. Affected monkeys provide a valuable model of Parkinson's disease. Since after IV MPTP the basal ganglia are affected bilaterally, there is no unaffected striatum in the same animal to serve as control tissue for comparison, and the neurological deficit is so severe that the animals require L-DOPA administration or intensive care to survive. The extremely short plasma half-life of MPTP suggested to us that infusion of MPTP into the internal carotid artery (ICA) would cause unilateral Parkinsonism.

Graded doses of MPTP, 0.2-1.2 mg/kg, were infused into the ICA of adult rhesus monkeys. Animals that received 0.4 to 0.8 mg/kg developed rigidity, bradykinesia, posturing deformity, involuntary movements, and resting tremor of the contralateral extremities and rotation toward the infused side. The extremities ipsilateral to the infusion were unaffected, which permitted the animals to move about freely and feed themselves without difficulty. Treatment with L-DOPA reversed all extrapyramidal signs. Infusion of L-DOPA and apomorphine reversed the direction of rotation. The brains of animals were analyzed for morphological, immunohistochemical and chemical changes. There was an extensive loss of dopaminergic neurons in nuclei A8, A9, and A10 of the substantia nigra and prominent loss of dopamine-containing nerve fibers in the basal ganglia on the infused side, whereas the corresponding tissues in the opposite half of the brain appeared normal. The dopamine and homovanillic acid content in the perfused putamen and caudate nuclei were less than 4% of those in the contralateral hemisphere.

Intracarotid injection of MPTP causes degeneration of the nigrostriatal pathway only on the side of infusion. The rate of rotation of affected animals can be quantified and used to assess the response to therapy. This primate model of Parkinson's disease should be useful for further investigation of Parkinson's disease and of the responses of normal, contralateral brain to unilateral nigrostriatal injury. It should also prove useful in the investigation of tissue implants in Parkinson's disease in primates.

#### Surgical Treatment of Medically Intractable Epilepsy - Conrad Kufta, M.D.

The aim of the surgical arm of the NINCDS epilepsy program is to develop surgical methods for more precise localization and safer resection of epileptogenic foci.

Several surgical procedures and methodologies have been introduced during the past 18 months in the ongoing attempt to better select and treat patients with medically intractable epilepsy. Specially designed and targeted sphenoidal electrodes are now being implanted to help localize epileptogenic foci which originate in the medial temporal lobe and in which electrical projection to the lateral temporal surface can lead to false localization of the source of epileptogenic activity.

Subdural surface electrodes which were designed at NIH, are being

implanted in selected patients to record closer to the cortical surface for longer periods than is possible with electrocorticography and to allow direct stimulation of cortical foci to discriminate motor, sensory, and language areas. Such localization helps the topographical identification of overlap between critical cortical areas and epileptic foci.

During surgery for focal epilepsy, depth electrodes are used to record from deep structures inaccessible by routine electrocorticography to identify areas of potential epileptic activity suggested and localized by the new preoperative diagnostic methods, PET scanning, MRI and electromagnetoencephalography (MEG).

In particular, MEG, which offers a more 3-dimensional localization of abnormal electromagnetic phenomena than EEG, was able to predict correctly the location of a focus causing complex partial seizures in a patient whose preoperative EEG (in retrospect) gave false localizing information. This focus was successfully extirpated and would have been missed if a standard temporal lobectomy had been performed. The new 7 (seven) channel MEG, which is now in place, promises even greater 3-dimensional localizing capability, and we are now studying focal motor seizures as well as complex partial seizures with this technique.

As abnormal foci which appear to cause clinical seizures are better defined, and more basic information about them is available pre-operatively, an attempt is made to identify chemical derangements in these focal areas. Norepinephrine, dopamine and DOPA were all shown to be significantly elevated in focal epileptiform tissue removed at surgery when compared to inactive (no active spiking observed during electrocorticography) and histologically normal tissue removed during the same resection. Epinephrine was not detectable in either focal or nonfocal tissue.

## **B. TISSUE CULTURE LABORATORY**

Marsha J. Merrill, Ph.D., Head

In the past year the Tissue Culture laboratory has studied three aspects of human glioma cell biology: 1) effects of differentiating agents on cultured glioma cells; 2) characterization of a vascular permeability factor produced by glioma cells, and 3) characterization of the interaction of insulin and insulin-like growth factors (IGF's) with glioma cells.

### **Effects of Differentiating Agents on Glioma Cells**

Differentiating agents (DA's) have been used successfully in model systems of certain types of cancer either individually to modify the tumorigenic phenotype, or synergistically with radiation or chemotherapeutic agents. This approach has not yet been tested with gliomas. We are now characterizing the effects of certain differentiation agents (sodium butyrate, cyclic AMP, dimethylformamide, and dimethylsulfoxide) on cultured glioma



cells. All of these agents decrease cell growth rate and alter cell morphology but none were effective in potentiating increasing cell killing by BCNU or AZQ, two brain tumor chemotherapy agents. Current efforts are now being directed toward the possibility that these agents may be useful in preventing or delaying tumor cell recovery following a chemotherapeutic insult. In an effort to better understand the effects of these differentiation agents at a more molecular level we are using two approaches. First, we have screened DA-treated vs. control cells against a panel of monoclonal antibodies raised against glioma cells. Second, we are examining the protein pattern of DA-treated vs. control cells by two-dimensional gel electrophoresis.

#### Vascular Permeability Factor Produced by Glioma Cells

We have determined that medium conditioned by cultured human glioma cells contains a substance capable of increasing vascular permeability. This vascular permeability factor (VPF) is probably at least partially responsible for the cerebral edema associated with brain tumors. VPF appears to be a protein which is distinct from other substances known to elicit edema. We are now in the process of purifying VPF so that we can characterize the molecule, the regulation of its production by the tumor cell, and the mechanism of action of VPF on the cerebral vasculature.

#### The Role of Insulin and Insulin-Like Growth Factors in Glioma Cells

Insulin and insulin-like growth factor (IGF) receptors have been detected in the human central nervous system. The role of IGF's in the nervous system is not well understood although IGF-I may be involved in the growth of the peripheral nervous system and IGF-II may play a role in glial cell growth and maturation in the brain. The role of insulin and IGF's in glioma cells has not yet been investigated. We have initiated studies to determine the nature of insulin and IGF receptors on human glioma cells derived from surgical specimens. Our results demonstrate the presence of receptors for IGF-I and IGF-II but not for insulin on cultured cells derived from human gliomas. Our findings suggest a role for IGF's in glioma cell metabolism. The functional significance of IGF receptors will be assessed by correlating the receptor binding of IGF's with the effects of IGF's on cell metabolism. These cultured cells may also provide a model system for study of the effects of IGF's on glial cells.

#### C. NEUROIMMUNOLOGY UNIT

Richard Webber, Ph.D., Head  
Elizabeth A. Grimm, Ph.D., Head (Departed NIH 5/1/86)

The Neuroimmunology Unit is dedicated to the study of cytotoxic lymphocytes. The immediate goal of this laboratory is the development and application of means to use cytotoxic lymphocytes for selective lysis of human

brain tumor cells. Studies are now in progress in rat and human tumor systems and are being pursued at the molecular, cellular, and in vivo levels. Dr. Grimm identified an in vitro method to generate tumor selective cytotoxic lymphocytes by activation with the lymphokine, interleukin-2. These lymphokine activated killer cells (LAK) provide a system that obviates the need for specific antigen recognition.

The first studies determined that lymphocytes from glioma patients respond to IL-2 and create LAK. Dr. Steven Jacobs was successful in creating LAK and has found that they kill both autologous (4/5 tests) and allogeneic (8/8) glioma cells in vitro. Lymphocytes cultured without IL-2 did not kill the autologous tumor (0/13). Normal cells were not lysed. Further experiments were designed to determine the nature of the epitope on glioma cells which render them susceptible to LAK killing. Treatment of the target cells with trypsin (0.1 or 0.01 mg/ml) did eliminate the ability of them to serve as targets for LAK. In contrast, a variety of enzymes and chemicals that alter cell surface carbohydrates had no effect. These results indicate that the moiety on glioma cells which is responsible for their susceptibility to killing by LAK is dependent on a protein determinant.

Because glioma patients receive Decadron<sup>R</sup>, which is believed to be a potent immunosuppressive agent, we studied the effect of hydrocortisone (decadron analog) in parallel with another agent, cyclosporine, to determine what effect these drugs had on the activation of LAK. We found that LAK activation was very sensitive to hydrocortisone ( $10^{-5}$ - $10^{-6}$ M) and resistant to cyclosporine (10ng-1ug/ml). Allospecific cytotoxic T lymphocytes (CTL) were prepared in parallel and their activation was found to be sensitive to cyclosporine and not to hydrocortisone. Although these results have led us to make further hypotheses concerning the mechanism of LAK activation, they were troubling because of the high levels of Decadron<sup>R</sup> received by the glioma patients. We therefore tested patient's peripheral blood lymphocytes (PBL), from those patients receiving up to 40 mg/day of Decadron<sup>R</sup>. It was found that patients receiving even the highest levels of Decadron<sup>R</sup> were able to make LAK cells. A second concern was that the Decadron<sup>R</sup> perhaps rendered the tumor cells of these patients resistant to killing by lymphocytes. Therefore, it was tested whether this drug affected the susceptibility of cultured glioma tumor to LAK lysis. Glioma cells were treated with hydrocortisone and were tested in parallel with untreated glioma cells as targets. Both were lysed equally well, indicating that the hydrocortisone does not affect the lysis of glioma by LAK cells.

Culture of human peripheral blood lymphocytes in purified natural or recombinant interleukin-2 in the absence of exogenous antigen or nitrogen causes the differentiation of non-lytic lymphocyte precursor cells into LAK. We have found that inhibitors of both proliferation and differentiation (gamma irradiation or mitomycin C) prevent LAK activity. Further studies were performed to elucidate the mechanism by which IL-2 alone induces proliferation and differentiation into killer lymphocytes. We found that the lysosomotropic agents  $\text{NH}_4\text{Cl}$ , chloroquine, or monensin, when preincubated with PBL will prevent LAK activation, indicating a role for the lysosomes in IL-2 processing. It was found that no IL-2 receptor molecule, defined by the monoclonal

antibody Tac--(generously supplied by Dr. Thomas Waldman, NCI) is apparant on LAK precursors. There, we have proposed that a nonreceptor mediated interaction of IL-2 with the cell is obligatory. Tac does appear on these cells after 24 hours in culture when they then need more IL-2 to proliferate. In collaboration with Dr. Anne Walter and Dr. Robert Blumenthal of the Lab. of Mathematical Biology (LMMB), NCI, we have undertaken a study of the means by which IL-2 might interact directly with the lipid bilayer of the lymphocyte cell membrane. Computer generated models of the IL-2 tertiary structure indicate that the IL-2 could aggregate into an ion permeable channel. These results have been conclusive in liposome models and we are now testing them with lymphocytes.

As part of our studies to define alternative means for LAK activation, we discovered that the *Streptococcus pyogenes* preparation OK-432, would activate PBL into LAK-like killer cells. (OK-432 has been used successfully in Japan in immunotherapy of intrapleural and ascites tumors). The OK-432 induced cytotoxic lymphocytes exhibited several properties identical to LAK cells. These included sensitivity of activation to irradiation or mitomycin C, dependence on IL-2, and relative resistance of the killer activity to leucine methyl ester.

Peter Brayton initiated molecular studies of LAK activation. We have performed one experiment in which we look for rearrangement of the T cell receptor beta gene elements and have found that they did not rearrange. To pursue these molecular studies in a controlled manner, LAK hybridomas have been prepared by fusing human LAK cells with a murine thymoma which is HAT sensitive. The fused products have then been cloned, grown into large quantities and are now being tested for LAK activity.

The study of the specificity of LAK lysis is continuing. Not only can we eliminate tumor cell susceptibility to LAK by proteases, but we have been able to create LAK sensitive targets from the normally resistant PBL. This has been performed by modifying proteins on the normal cell surface with a hapten, trinitrophenol (TNP). Not only are TNP-PBL lysed by LAK but cold target inhibition studies indicated that lysis is inhibited by fresh tumor cells (7/7 experiments) and that tumor lysis is inhibited by TNP-PBL (5/7 experiments). Additionally, it was found that allogeneic tumors totally inhibited lysis of autologous tumors in other cold target studies (3/3 experiments). These results demonstrate the lytic activity expressed by LAK is not HLA restricted, is not limited to tumor cells, and is nonspecific as indicated by the cross reactive recognition of multiple target cell types.

Because of the efficiency and apparent tumor selectivity of LAK lysis, we performed a clinical trial of direct intraoperative intracerebral injection of LAK cells in glioma patients. Prior to the initiation of these phase I clinical studies it was essential to determine whether or not LAK cells lysed normal brain tissue. Therefore, we adopted the rat glioma model, using the 9L tumor. Spleen cells from Fisher rats were cultured ( $10^6$ /ml) with or without IL-2 and then tested for their ability to lyse the syngeneic 9L glioma cells in vitro in using the standard 4 hour chromium release methods, identical to that used in the human. We found that the IL-2 culture did generate rat LAK

that would lyse tumor but no comparably prepared normal rat brain tissue. As in the human, rat lymphocytes cultured in the absence of IL-2 did not generate cytotoxicity. These results indicate that LAK cells lyse glioma tissue but not normal brain.

The fate of LAK cells following injection into the brain was also pursued in the rat system. LAK labelled with indium 111 were injected into the brain of normal rats. The animals were then sacrificed and the brain removed, sectioned, and autoradiography performed. Our results found that LAK remained localized to the injection site for as long as 72 hours later.

Therefore, a clinical trial (protocol #84-N-238) was initiated ("Immunotherapy of brain tumors by interleukin 2 and interleukin 2 activated autologous lymphocytes"). We injected, in increasing doses, either LAK ( $10^8$ ,  $10^9$  or  $10^{10}$ ) cells or IL-2 ( $10^4$ ,  $10^5$ ,  $10^6$  units) into the brain tissue surrounding the cavity left following debulking of tumor. Thirteen patients were treated. No obvious signs of toxicity were observed. These patients received up to  $10^6$  units of IL-2 and up to  $1 \times 10^8$  LAK cells.

#### D. BIOCHEMISTRY LABORATORY

Richard J. Youle, Ph.D. - Head

##### Monoclonal Antibody Mediated Killing of Tumor Cells

The Unit of Biochemistry, headed by Dr. Richard Youle, is studying the use of monoclonal antibodies to kill disease-causing cells. Monoclonal antibodies which selectively bind tumor cells can be generated, but alone are usually not cytotoxic to the tumor. Toxic proteins such as ricin and diphtheria toxin can be chemically linked to monoclonal antibodies. The new hybrid molecules bind tumor cells via the antibody moiety and then kill the cells via the toxin moiety. The toxins used are enzymes that catalytically inactivate protein synthesis in target cells with only one or two molecules in the cytoplasm killing a cell. However, the non-target cell toxicity of the toxins must be blocked with excess lactose to prevent toxin binding and this currently limits this approach to in vitro applications. The cell-type-specific reagents have immediate clinical application in vitro in bone marrow transplantation where T cell depletion improves allogeneic transplantation. The Unit of Biochemistry is supplying these reagents for clinical trials in bone marrow transplantation at the University of Minnesota. Twenty-four patients have now been treated with immunotoxin purged bone marrow as the sole prophylaxis for graft-versus-host disease. Comparing the outcomes with historic controls treated post-transplant with methotrexate, several conclusions can be drawn. The patients had a milder course as evidenced by a significantly shorter hospitalization. Engraftment of donor marrow occurred with a shorter time until leucocyte generation and no severe graft-versus-host disease was seen. Clinical trials of immunotoxin treatment of bone marrow are continuing to increase the patient population and thus the statistical significance of the apparent benefits.

The major goal of the laboratory is to develop immunotoxins which will

selectively kill tumor cells in vivo. Currently the limiting steps for antibody-toxin hybrids are (1) the entry of the toxin molecule into the cell and (2) in vivo access of the drug to the tumor cells.

Several new approaches have been successfully applied in vitro and in vivo in the past year.

A) To promote access of monoclonal antibody-toxin conjugates to tumors we have focused on tumors localized in body compartments such as the brain and the peritoneal cavity. The brain may be an optimal compartment for antibody modulation of cell function.

1. We developed an animal model of carcinomatosis meningitis. Human leukemia cells, CEM, were injected intracerebrally or ( $10^5$  cells) into the CSF via the cisternum magnum, into nude rats. Ten to fourteen days later animals die due to the brain tumor.
2. We have localized a monoclonal antibody to the solid tumor in the cerebral tumor model. The surprising and encouraging result is that the antibody not only bound to CSF borne cells but also bound cells several millimeters into the brain parenchyma. We are currently testing therapy of this tumor with antibody-toxin conjugates.
3. We have synthesized an antibody-toxin conjugate that binds the transferrin receptor and binds human brain tumor cells 5 times more than normal human brain cells. In vitro the antibody-ricin conjugate specifically kills tumor cells via the tumor-associated antigen.
4. We have treated a peritoneal tumor with peritoneally injected antibody-toxin conjugate and achieved 25% long-term survivors and a very significant extension in mean survival time. We are currently considering adapting this procedure to clinical testing in humans with ovarian cancer.

B) To improve entry of immunotoxins into cells we have modified toxins chemically, molecular biologically and with monoclonal antibodies. We are studying the structure-function relations of the toxin molecule and the cell biology of toxin internalization and membrane penetration.

1. We raised a panel of monoclonal antibodies (MoAb) to study the various activities of ricin. One antibody binds the lactose binding site on ricin and affects immunotoxins in vitro like lactose. In vivo the MoAb protects mice from ricin toxicity like asialofetuin. Monoclonal antibodies stay in circulation for days and should be even better than asialofetuin for blockage of ricin B chain. We are initiating in vivo therapy of cancer in animal models using intact ricin immunotoxins plus the anti B chain monoclonal antibody against the ricin binding site.
2. Toxins may be best adapted for tumor specific toxicity by alterations of amino acid sequence at the gene level. To begin improving

immunotoxins at the gene level we have worked with the prokaryotic toxin, diphtheria toxin. Intact diphtheria toxin was linked to a monoclonal antibody specific for human T cells and was found to specifically kill target cells at  $10^{-12}$ M. The rate of specific killing was 10 fold faster than previously reported immunotoxins. This model system was then used to study cloned fragments of diphtheria toxin. In collaboration with Dr. Larry Greenfield, who has cloned diphtheria toxin (DT), we deleted the C-terminal 15KD region of the toxin which had previously been shown to include the cell surface binding site. This left the toxin A subunit plus a 17 KD fragment of DT B chain thought to facilitate transmembrane transport. When linked to monoclonal antibodies, this truncated DT was 100 fold more toxic than DTA chain linked to antibody and the toxicity was blocked by excess antibody. The cloned DT fragment was 1000 times less toxic to guinea pigs than the native toxin. Therefore, the fragment of DT B chain included by cloning increased target cell toxicity more than non-target cell toxicity indicating that separation of B chain entry functions from binding was accomplished to some degree. Comparing intact DT linked to monoclonal antibody with the cloned fragment of DT showed the C-terminal fragment of DT further increased antibody mediated toxicity 100 fold. We are currently studying conjugates of 3 point mutants of diphtheria toxin linked to antibodies in attempts to include the 100 fold activation by the C terminus of DT while omitting regions causing non-target cell killing.

3. We are studying the mechanism of toxin entry into cells to allow improved design of tumor-specific reagents. Toxins like ricin and diphtheria toxin bind the cell surface, are endocytosed, then cross the membrane surrounding the endocytotic vesicle to reach the cytosol where they inactivate protein synthesis. The rate limiting step in toxin and immunotoxin activity is transport across the membrane to the cytosol. How and where this transport step occurs is unknown. To investigate this question we have used hybridoma cells which secrete monoclonal antibodies that block ricin toxicity. We have found that these cells are themselves resistant to ricin because of the antibody they synthesize. We found that the resistance was not caused by extracellular or cell surface antibody but by antibody within the cell in route to secretion. This means that ricin must pass through the protein secretory pathway, comprising the endoplasmic reticulum, golgi apparatus, and secretory vesicles, before entering the cytosol. This new approach may be applied to study other toxins, hormones and macromolecules which enter cells by receptor mediated endocytosis.

### Programmed Cell Death in the Nervous and Immune System

Our laboratory has begun a project to study the mechanism and physiologic role of programmed cell death. In both the nervous system and the immune system massive numbers of cells die during normal development. In the spinal cord for example, over half the neurons die during fetal development. The immune system also has large cell loss at precise stages in development such

as thymocyte death and thymus involution at puberty. The physiologic role and the biochemical mechanism of programmed cell deaths is unknown. Understanding the mechanism and regulation of such physiological cell deaths may shed light on neurodegenerative diseases and immunodeficiency disorders. One rare hereditary disease, Ataxia telangiectasia, demonstrates the relationship between cell death in the nervous and immune systems. This disease results from a single recessive gene and is characterized by Purkinje cell death and premature thymus involution. Patients are ataxic but usually die from infections resulting from a pronounced immunodeficiency.

In our initial study we found that thymocytes could be induced to die by the same signal that stimulates mature T cells to proliferate. RNA and protein synthesis inhibitors block new gene expression and block the programmed cell death. This is consistent with a new model of immune system self-tolerance that states that potentially auto-reactive cells are induced to die by cross-linking the T cell receptor early in development, the same signal that induces mature T cells to react against foreign antigens.

We are extending our studies into the nervous system by examining the mechanism of thymocyte death in various ataxic mutant mice and by examining the mechanism of glutamate induced neuron death in vitro and in vivo.

### E. CELLULAR BIOLOGY LABORATORY

Joseph Bressler, Ph.D. - Head

Work in our laboratory continues to investigate mechanisms by which oncogenic signals and biologically active molecules influence glial differentiation. We have shown that various types of tumor promoters as well as other molecules which activate protein kinase C inhibit the expression of an oligodendroglial specific property in rat glioma cells. In addition, we demonstrated that sodium butyrate (NaBu) inhibited the expression of a glial specific property, S-100 levels in both rat and human glioma cells, but did not influence the expression of glial fibrous acidic protein (GFAP) in a human glioma-derived cell line. Other short chain fatty acids were active, though NaBu exhibited the highest potency. One possibly mechanism in which NaBu and protein kinase C activators may work is by decreasing cAMP levels. We have explored this possibility, and have observed that both types of molecules inhibit the beta-adrenergic and forskolin response in C6 rat glioma cells. Phosphodiesterase levels were not altered by NaBu or by the protein kinase C activators.

#### Growth Factor Stimulation of Astrocytes

Our laboratory has also been concerned with the effect that growth factors have on glial cells. Previously, we demonstrated that platelet derived growth factor (PDGF) could serve as a chemotactic signal for rat astrocytes. During the process of chemotaxis, at least two changes take place 1) a change in cell shape and 2) proteins are phosphorylated. Both processes

have been studied. A change in shape may be mediated by alterations in intermediate filament and actin levels. We have shown that GFAP, actin and vimentin levels decreased when rat astrocytes are grown in reaggregating cultures. GFAP and vimentin levels will also decrease if they are plated on a collagen matrix. Both types of culture conditions decrease the amount of cell spreading. We showed that GFAP, vimentin and actin levels decrease when human glioma cells are grown on a polyhema matrix. Again, polyhema causes the cells to change their shape.

We studied proteins which were phosphorylated after phorbol ester stimulation since phorbol esters and PDGF activate the protein kinase. After optimizing conditions, we found three proteins which exhibited between 50-150% more phosphate. The molecular weight of these proteins are 85,000, 60,000 and 25,000.

### Neoplastic Transformation of Human Glial Cells

An additional phenomenon that we have been concerned with is the neoplastic transformation of a human glial cell line in culture. This cell line, derived by Dr. Gene Major, Infectious Disease Branch, NINCDS, is from human fetal brain cells transfected with a SV40 mutant which lacked the point of origin. The transformed cells lost the SV40 T antigen but were tumorigenic in nude mice and were anchorage independent. Though phorbol esters were mitogenic for this line, they did not influence the transformation process.

### Effect of Biological Modifiers on Glial Differentiation

For the past several years we spent considerable amount of time trying to determine if any of the known biological modifiers promote glial differentiation. Unfortunately, many of the drugs inhibited differentiation in the cell lines studied. Another approach to these studies is to develop a toxin which effects cells that express a specific differentiated trait. Recently, it has been shown that alpha-aminoadipic acid (a glutamate analog) is a specific toxin for glial cells and allows neurons to remain intact. We propose to study whether glioma cell lines are more sensitive to alpha-aminoadipic acid than neuroblastoma or other non-neural derived cell lines. We are interested in using this drug to treat nude mice with implanted human glioma tumors.

### Growth Factor Stimulation of Astrocytes

A diverse array of growth factors have been shown to stimulate astrocyte growth. It is unclear whether all subpopulations of astrocytes are sensitive to the same factors. It is also not known if each factor stimulates similar genes. To characterize subpopulations we will treat primary cultures of rat astrocytes with different growth factors, and then treat them with antisera against these factors. This will be followed by secondary antibody linked to fluorescein to determine the per cent of cells which bind the individual



factors. We also plan to determine doses of each factor needed to stimulate astrocytes, and then determine if the effects of these factors are additive at saturating doses. Finally we plan to obtain iodinated probes of these growth factors and determine, by competition studies, whether these factors bind to the same receptor.

Genes activated after growth factor stimulation will be studied by isolating RNA from stimulated astrocytes. The RNA will then be hybridized with known proto-oncogenes to determine activation.

We will also continue to study proteins which are phosphorylated after phorbol ester stimulation. We are interested in determining the location of these proteins in the cell.

### Neoplastic Transformation of Human Glial Cells

DNA and RNA from transformed and non-transformed human glial cells will be hybridized to known oncogenes in order to determine oncogene activation. Within this closed system, we will be able to determine mechanisms important in the ontogeny of human gliomas.

### F. ELECTRON MICROSCOPY LABORATORY

Gregory Hook, Ph.D. - Head

The Electron Microscopy (EM) Laboratory studies involve structural aspects of glioma killing by interleukin 2 (IL-2) stimulated peripheral blood mononuclear cells (LAK). LAK have been shown to kill glioma cells in vitro and may be useful in treating brain tumors clinically.

Very little is known about the mechanism of glioma killing by LAK cells. LAK cells are a heterogenous cell population composed of different cell types. It is not known which subpopulation within LAK are the killer (effector) cells. A subpopulation of particular interest is the Tac antigen-positive LAK cells because the Tac antigen is closely associated with the IL-2 receptor and is specific for activated T cells. For these and other reasons, the Tac positive LAK cells have been postulated to be the effector cells within the LAK population.

Two of the goals of the EM laboratory are: 1) the ultrastructure of glioma killing by LAK and 2) determining if Tac positive LAK are the effector cells. To study glioma killing by LAK, an established glioma cell line and LAK from normal adult volunteers are used as an in vitro model. The mixed culture of glioma and LAK cells react, non-adhering LAK cells are removed, adhering cells are labeled with anti Tac and examined by fluorescence light microscopy, scanning electron microscopy and transmission electron microscopy. Immunofluorescence light microscopy and immunogold electron microscopy methods have been developed to specifically label Tac-positive LAK cells for light and electron microscopic examination. Individual Tac-positive LAK cells are

identified by indirect labeling whereby the Tac antibody selects the Tac positive cells and a secondary fluorescent molecule or gold sphere label labels the anti Tac.

Immunofluorescence light microscopy shows that Tac-positive LAK cells are a subpopulation of the LAK cells. Mixed glioma and LAK cells show that Tac-positive and negative LAK cells bind glioma cells but it is not possible to determine which LAK cells are killing the glioma cells from the light microscopic preparation. Immuno-transmission and scanning electron microscopy experiments of LAK and glioma cells are currently being conducted and preliminary results show Tac-positive and negative LAK cells attach to glioma cells.

This is a collaborative project with Drs. Elizabeth Grimm, David Barba and Richard Webber of the Surgical Neurology Branch and Dr. Linda Muul of the Surgery Branch, National Cancer Institute. The antibody to the Tac antigen is kindly supplied by Dr. Thomas Waldman of the Metabolism Branch and the immunolabeling method was developed in collaboration with Dr. Mark Willingham of the Laboratory of Molecular Biology, National Cancer Institute.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS02454-06 SN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biological Studies of Human Pituitary Tumors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Edward H. Oldfield, M.D., Acting Chief, Principal Investigator      SN NINCDS Surgical Neurology Branch, NINCDS		
COOPERATING UNITS (if any) Developmental Endocrinology Branch, NINCDS Diagnostic Radiology, CC		
LAB/BRANCH Surgical Neurology Branch		
SECTION Clinical Neurosurgery Section		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The influence of the hypothalamic releasing factors CRF and GRF on the hormonal secretion of pituitary adenomas has been determined <u>in vitro</u> and correlated with the patient's response <u>in vivo</u>. These studies indicate that the pituitary tumors causing Cushing's disease, Nelson's Syndrome and acromegaly are responsive to their appropriate releasing factor. We are investigating the potential of using releasing factors conjugated to toxic proteins to effect cytotoxicity of pituitary tumors <u>in vitro</u>.</p> <p>We have investigated the use of venous sampling to aid in the diagnosis and treatment of patients with Cushing's Syndrome. Our results, which now include 89 patients with Cushing's syndrome, suggest that the procedure will be of significant benefit in: 1) establishing the diagnosis of Cushing's disease and 2) determining preoperatively the site of pituitary microadenomas.</p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02687-02 SN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanism of Interleukin-2 Activation of Cytotoxic Lymphocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Rick Weber, Ph.D.	Principal Investigator	SN NINCDS
Elizabeth A. Grimm, Ph.D. (departed 5/86)	Senior Investigator	SN NINCDS
Peter Brayton, Ph.D.	Senior Staff Fellow	SN NINCDS
Masato Yagita	Fogarty Fellow	SN NINCDS
Debbie J. Wilson	Technician	SN NINCDS
Barbara Ikejiri	Technician	SN NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Surgical Neurology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  Activation of cytotoxic lymphocytes is a complex and poorly understood process. Two immunosuppressive drugs, Cyclosporine A (CsA) and Hydrocortisone (Hy) were examined in parallel for their effect on the generation of cytolytic lymphocytes. Peripheral blood lymphocytes (PBL) were stimulated with allogeneic cells to produce allospecific CTL, or with purified recombinant IL-2 (R IL-2) to activate lymphokine-activated killer cells (LAK). Both CTL and LAK activity were measured in a 4-hour chromium release assay after 7 days of activation. Lysis by CTL was tested against stimulator PBL (not blasts) and LAK against fresh sarcoma tumor cells. At pharmacologic doses, CsA inhibited only CTL generation, while HY inhibited only LAK. LAK activation is believed to occur by interaction of IL-2 with the precursor cell, via a non-receptor mediated interaction. We are attempting to define the exact means of interaction, and then pursue the early events leading to the expression of cytotoxic activity.		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02686-02 SN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunotherapy of Brain Tumors by Interleukin-2 and Activated Lymphocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Rick Weber, Ph.D.	Principal Investigator	SN NINCDS
Elizabeth A. Grimm, Ph.D. (departed 5/86)	Senior Investigator	SN NINCDS
B. Holcomb	Student Volunteer	SN NINCDS
Paul Kornblith, M.D. (departed 6/86)	Chief, SNB	SN NINCDS
Steven Jacobs, M.D.	Senior Staff Fellow	SN NINCDS
Debbie Wilson	Technician	SN NINCDS
Gilbert Melin	Student Volunteer	SN NINCDS
Catherine Parham	Student Volunteer	SN NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Surgical Neurology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Culture of brain tumor patient peripheral blood lymphocytes (PBL) with recombinant interleukin-2 (IL-2) results in the activation of lymphokine activated killer cells (LAK) with the capacity to lyse autologous and allogeneic glioblastoma. PBL obtained from brain tumor patients were cultured with or without IL-2 for three to seven days and then tested for their ability to lyse target cells in a 4-hour chromium release cytotoxicity assay. PBL were drawn one to two weeks following operative tumor debulking. Cells used as targets included fresh brain tumor cells obtained at the time of craniotomy, fresh brain tumor grown from one to three weeks in tissue culture, fresh autologous PBL and allogeneic glioblastoma grown in tissue culture.</p> <p>Brain tumor patient PBL cultured without IL-2 did not significantly lyse autologous or allogeneic glioblastoma. However, when these PBL were cultured with IL-2, LAK was generated which produced marked lysis of autologous as well as allogeneic tissue culture glioblastoma in all of eight cases. Significant lysis of autologous fresh tumor by patient LAK was observed in four of five experiments. By contrast, patient's LAK did not kill autologous normal PBL. The ability to generate LAK was not influenced by patient age, previous therapy or the administration of steroids.</p> <p>Eleven patients have received either IL-2 or LAK cells into their brain tissue at the time of surgical debulking of confirmed glioma. No toxicities have been observed.</p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02685-02 SN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Analysis of Specificity of LAK-Mediated Target Cell Destruction</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Rick Weber, Ph.D.	Principal Investigator	SN NINCDS
Elizabeth A. Grimm, Ph.D. (departed 5/86)	Senior Investigator	SN NINCDS
Steven K. Jacobs	Senior Staff Fellow	SN NINCDS
Barbara Ikejiri	Technician	SN NINCDS
Gilbert Melin	Student Volunteer	SN NINCDS
COOPERATING UNITS (if any)		
LAB/BRANCH Surgical Neurology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.75	PROFESSIONAL: 1.75	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           Culture of human peripheral blood lymphocytes (PBL) in purified natural or recombinant interleukin-2 in the absence of exogenous antigen or nitrogen causes the differentiation of non-lytic precursor cells into lymphokine-activated killers (LAK). A titration of purified Jurkat IL-2 (BRMP, FCRC, NIH) IL-2 showed that the relatively low concentration of 5 U/ml was optimal for LAK activation. The spectrum of target cells susceptible to LAK lysis in a 4-hour chromium-51 release assay includes fresh NK-resistant tumor cells and trinitrophenol (TNP) modified autologous PBL. Unmodified PBL are not lysed. Cold target inhibition studies indicated the LAK lysis of autologous TNP-PBL is totally inhibited by fresh tumor cells, and that tumor lysis is inhibited by TNP-PBL. Additionally, allogeneic tumors totally inhibit lysis of autologous tumor cells in other cold target studies. These results demonstrate that the lytic activity expressed by LAK is not HLA restricted, is not limited to tumor cells, and is "polyspecific" as indicated by the cross-reactive recognition of multiple target cell types in these cold target inhibition studies.         </p> <p>           The mechanism by which LAK effector cells mediate tumor cell destruction is unknown. Lysis occurs rapidly at 37°, and 4 hours of incubation is optimal. The mechanism is neither an antibody-mediated cytotoxicity (ADCC) nor merely lectin dependent cytotoxicity (LDCC). The report of successful adoptive therapy in mouse systems with LAK provides the basis for proposing the LAK is a biologically relevant system in which to further examine the mechanism and specificity of cell-mediated cytotoxicity.         </p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02673-02 SN
PERIOD COVERED October 1, 1985 through December 31, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Monoclonal Antibodies Linked to Ricin for Use in Human Bone Marrow Transplantation</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Richard J. Youle, Ph.D. Joseph Dalton	Principal Investigator Chem. Lab. Technician	SNB/NINCDS SNB/NINCDS
COOPERATING UNITS (if any) University of Minnesota, Department of Laboratory Medicine National Cancer Institute, Immunology Branch, DCBD National Institute of Mental Health, Laboratory of Molecular Biology, LMB		
LAB/BRANCH Surgical Neurology Branch, NINCDS		
SECTION Biochemistry Unit		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             Bone marrow transplantation is the treatment of choice for high risk leukemia, aplastic anemia and other immunodeficiency disorders. It is also being used for therapy of other radiation sensitive tumors such as neuroblastoma and for inherited enzyme deficiency disorders. The limiting complication is graft-versus-host disease (GVHD) caused by mature T cells in the donor marrow recognizing histocompatibility differences between donor and host. Studies in animals and humans have shown that removal of mature T cells from the donor marrow while preserving the pluripotent stem cells prevent GVHD.           </p> <p>             Monoclonal antibodies linked to toxic proteins can specifically kill cells based on cell surface antigen differences. We have developed a panel of T cell selective toxins which kill up to 5 logs of T cells at concentrations non-toxic to human stem cells.           </p> <p>             We have begun clinical trials of these reagents for 1) prevention of GVHD in MHC matched bone marrow recipients; 2) prevention of GVHD in MHC mismatched BMT. Twenty three patients have now been treated. Ten patients transplanted for high-risk leukemia with major HLA matched sibling marrow are now evaluable. The antibody-ricin conjugate showed no toxicity to the patients. Comparing antibody-toxin treatment with conventional post-transplant methotrexate prophylaxis the hospitalization stay was significantly shorter. Engraftment of donor marrow occurred in all patients and 8 out of 10 showed predominantly donor lymphoid cells 30 days post-transplant. No cases of severe GVHD were observed. Continued clinical trials are underway to increase population size to statistically significant levels to compare GVHD incidence of immunotoxin vs. conventional protocols.           </p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02674-02 SN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Monoclonal Antibody-Toxin Conjugates for Tumor Therapy In Vivo</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Richard J. Youle, Ph.D.	Principal Investigator	SN NINCDS
Virginia Gray Johnson, Ph.D.	Staff Fellow	SN NINCDS
John Zovickian, M.D.	Medical Staff Fellow	SN NINCDS
Charles Riedel, M.D.	Medical Staff Fellow	SN NINCDS
Joseph Dalton	Chem. Lab Technician	SN NINCDS
COOPERATING UNITS (if any) Laboratory of Immunology, NIAID Cetus Corporation		
LAB/BRANCH <u>Surgical Neurology Branch</u>		
SECTION <u>Office of the Chief</u>		
INSTITUTE AND LOCATION <u>NINCDS, National Institutes of Health, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Monoclonal antibodies selectively bind tumor cell differentiating antigens <u>in vitro</u> and <u>in vivo</u>. Natural effector mechanisms often do not mediate killing of monoclonal antibody bound cells so we have devised methods of linking extremely toxic proteins to the antibodies to selectively kill tumor cells.</p> <p>Two methods of coupling toxic proteins, like ricin to antibodies, have been used to kill antigen positive cells <u>in vitro</u>. Ricin has two subunits, the A subunit blocks protein synthesis when in the cytosol and the B subunit binds galactose groups on all cell surfaces but also facilitates the transport of ricin A chain to the cytosol. 1) Linkage of the ricin A chain to antibodies yields reagents with low non-target toxicity but target cell toxicity too slow for <u>in vivo</u> applications; 2) Linkage of intact ricin to antibodies results in very potent target cell toxicity but the non-target cell killing must be prevented by a ligand which blocks ricin B chain binding to cells. This has limited its application to <u>in vitro</u> situations where 100 mM lactose can block ricin binding.</p> <p>We are testing several new approaches to apply immunotoxins <u>in vivo</u>. 1) Cloning of toxins then altering their structure at the gene level to decrease non-target cell toxicity; 2) Chemical modification of ricin to determine the location of the ricin galactose binding site and to possibly improve efficacy of ricin linked to antibodies; 3) Develop new ways to block the non-target cell toxicity of ricin <u>in vivo</u>. We have discovered a monoclonal antibody which blocks the ricin galactose binding site similar to lactose. Preliminary <u>in vivo</u> trials in guinea pigs show over a 2 fold extension of survival time with intact ricin immunotoxins with no toxicity to animals. 4) Intrathecal administration of monoclonal antibodies allows a new way to image brain tumors. We are now testing intrathecal administration of immunotoxins for therapy of brain tumors.</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02695-02 SN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Loss of Differentiation Function in Transformed Glial Cells</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between;"> <span>Joseph Bressler, Ph.D.</span> <span>Principal Investigator</span> <span>SN NINCDS</span> </div>		
COOPERATING UNITS (if any)  Laboratory of Cellular Carcinogenesis and Tumor Promotion, LCCTP, DCE, NCI		
LAB/BRANCH Surgical Neurology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>Previous work from our laboratory suggest that oncogenic signals modulate glial differentiation. For example, activation of the protein kinase C will cause the C6 rat glioma cell line to lose the ability to increase glycerol phosphate dehydrogenase levels after glucocorticoid stimulation. One possible mechanism which would allow this to occur is by inhibiting the cell from increasing cAMP levels. We have found that elevated diacylglycerol levels (the natural ligand for the protein kinase C) causes inhibition of the beta-adrenergic and forskolin stimulated increased in cAMP levels in whole cells. This effect was not due to changes in phosphodiesterase levels or in beta-adrenergic receptor levels. We have also found that sodium butyrate inhibits the cell from increasing S-100 levels and this drug also interferes with the forskolin and beta-adrenergic response without influencing the phosphodiesterase levels. Therefore one common mechanism by which a cell is made to alter its differentiating program is through cAMP dependent mechanisms.</p> <p>We have also attempted to discern how cell shape alters the expression of differentiation. This is important since tumor cells do not spread or adhere as well as nontransformed cells. Primary cultures of astrocytes do not synthesize as much glial fibrous acidic protein (GFAP), vimentin or actin when grown in suspension, or on collagen matrices. Human glioma cells also synthesize less of these proteins when grown as round cells. Therefore shape is an important factor in the expression of differentiation.</p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 NS 02696-02 SN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Intraarterial Chemotherapy Combined with Extracorporeal Drug Removal		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Edward H. Oldfield, M.D.  Dr. Robert Dedrick  John Doppmann, M.D.	Principal Investigator, SNB, NINCDS  Bioengineering and Instrumentation Br. Division of Research Services, NCI  Diagnostic Radiology Department, CC	
COOPERATING UNITS (if any) Bioengineering and Instrumentation Branch, Division of Research Services Diagnostic Radiology Department, Clinical Center		
LAB/BRANCH Surgical Neurology Branch		
SECTION Clinical Neurosurgery Section		
INSTITUTE AND LOCATION NINCDS, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>One of the basic tenets of anticancer chemotherapy is that increased tumor exposure to drug should result in increased tumor response. One method currently being used to increase drug exposure of malignant brain tumors is by intracarotid infusion. Although intracarotid infusion increases drug delivery, systemic toxicity, not brain toxicity, frequently limits the tolerable dose. A means of extracting the drug from the blood after one passage through the brain, and before the high concentration of drug reaches the general circulation, should allow dose escalation to levels which provide increased tumor response. The following have been demonstrated.</p> <p>1) A pilot study of 4 patients treated with intracarotid BCNU during extracorporeal hemoperfusion of the jugular venous blood demonstrated that the drug exposure of the body could be reduced by 56-87% by channeling the blood extracorporeally at 300 ml/min through a hemoperfusion cartridge for drug extraction. The pharmacokinetic advantage (ratio of brain exposure to body exposure) compared to intravenous infusion of the same dose was 21-55:1.</p> <p>2) Drug streaming during intracarotid delivery results in maldistribution of the infused agent with the potential of delivering very high (toxic) concentrations of drug to some regions of the brain, while other areas receive minimal drug. Drug streaming is probably the cause of focal encephalomalacia following intracarotid infusion of BCNU.</p> <p>3) A dye dilution study of intracarotid infusion of indocyanine green demonstrated that the recovery of substances from the jugular blood after intracarotid infusion is linearly related to the rate of aspiration of the blood from the jugular blood. This suggests that the amount of an injected drug which can be removed from blood before the blood is returned to the body is dependent on the rate at which blood is withdrawn from the jugular vein.</p> <p>4) Extracorporeal circulation of the jugular blood for drug removal during intracarotid infusion of cis-platinum resulted in tumor exposures 5-15 flow greater than the exposure of the remainder for the body.</p>		

## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02697-02 SN

## PERIOD COVERED

October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Metabolic Differential Between Brain and Brain Tumor with Thiopental

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Edward Oldfield, M.D., Acting Chief

Principal Investigator, SNB/NINCDS

## COOPERATING UNITS (if any)

Giovanni DiChiro, M.D., NIS, ODIR, NINCDS

## LAB/BRANCH

Surgical Neurology Branch

## SECTION

Clinical Neurosurgery Section

## INSTITUTE AND LOCATION

NINCDS, National Institutes of Health, Bethesda, Maryland 20892

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL:

0.3

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project involves FDG-PET scanning of patients who have brain tumors before and during deep barbiturate general anesthesia. The results indicate that a profound reduction of cerebral glycolytic activity can be achieved with a level of anesthesia which produces burst-suppression EEG activity. Gliomas, however, have only a minimal change in glycolytic rate under the barbiturate anesthesia. The results also indicate that lower grade lesions, which are not visible on PET scans performed with the patient awake, become visible as background synaptic activity is suppressed with barbiturates. The true extent of growth into the surrounding tissue by higher grade lesions can be better appreciated when background activity is reduced under the barbiturate anesthesia. This work provides evidence that barbiturates may allow a "reverse contrast enhancement" of lesions with decreased neuronal activity. This phenomenon may provide a basis for development of specific antitumor therapy. The technique also may be valuable in studying other pathological processes such as degenerative diseases, epilepsy, movement disorders, cerebral infarction and head injury.

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02706-01 SN
PERIOD COVERED October 1, 1985 - September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effects of Differentiation Agents of Human Glioma Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;">           Paul L. Kornblith, M.D. (Departed 6/86)            Marsha J. Merrill, PH.D.            Calvin Hawkins            Andrew Fields         </div> <div style="width: 45%;">           Senior Investigator            Staff Fellow, Principle Investigator            Technician            Technician         </div> </div>		
COOPERATING UNITS (if any)  Dupont Company, Glenolden, PA		
LAB/BRANCH Surgical Neurology Branch, NINCDS		
SECTION Tissue Culture Unit		
INSTITUTE AND LOCATION NINCDS		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: --
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>Recent advances have been made in the use of differentiating agents in the treatment of certain cancers. These agents have been used both as adjuncts to traditional chemotherapy and as inducers of terminal differentiation. Although the most promising results have been in the field of leukemia research, progress is also being made with certain solid tumors. Our aims have been to examine the effect of certain differentiating agents on cultured glioma cells, to determine the interaction of these agents with standard chemotherapeutic agents, and to better characterize the "differentiated" state induced by these agents.</p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 NS 02707-01 SN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>The Role of Insulin and Insulin-Like Growth Factors in Glioma Cells</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;">           Marsha J. Merrill, Ph.D.            Nancy A. Edwards         </div> <div style="width: 50%;">           Staff Fellow, Principle Investigator            Technician, SNB/NINCDS         </div> </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH <u>Surgical Neurology Branch</u>		
SECTION <u>Tissue Culture Unit</u>		
INSTITUTE AND LOCATION <u>NINCDS, National Institutes of Health, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS: <u>0.5</u>	PROFESSIONAL: <u>0.5</u>	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>             Insulin and insulin-like growth factor (IGF) receptors have been detected in normal human brain. However, the role of the IGF's in the brain is not well understood. Although considered to be primarily a fetal growth factor, IGF-II levels continue to be detected at high levels in the CNS of adults, although the functional significance of this observation is unknown. Similarly, what role the IGF's play in brain tumors is unknown. We are initiating studies on normal and malignant brain tissue and in cell cultures derived from these tissues in order to begin to understand the physiologic significance of this family of growth factors in the human brain. Preliminary experiments have detected the presence of IGF but not insulin receptors on cultured glioma cells, suggesting that IGF's may play a role in glioma cell growth.           </p>		

<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER ZO1 NS 02708-01 SN
PERIOD COVERED October 1, 1985 through September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Vascular Permeability Factor Produced by Human Glioma Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Edward Oldfield, M.D., Acting Chief Marsha J. Merrill, Ph.D. Ross R. Moquin, M.D.	Principal Investigator, SNB/NINCDS Staff Fellow, SNB/NINCDS Staff Fellow, SNB/NINCDS	
COOPERATING UNITS (if any)		
LAB/BRANCH <u>Surgical Neurology Branch</u>		
SECTION <u>Tissue Culture Unit</u>		
INSTITUTE AND LOCATION <u>NINCDS, National Institutes of Health, Bethesda, Maryland 20892</u>		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             Cerebral edema associated with malignant brain tumors often causes neurologic deficits and increased intracranial pressure, which contribute to the morbidity and mortality of the neoplasm. We have determined that the conditioned medium of glioblastoma-derived cell cultures contains a substance capable of increasing the vascular permeability in a bioassay which measures the induction of capillary permeability in normal skin. The elicitation of this response by conditioned medium correlates with the edema observed clinically in patients from which these tumor cell cultures were derived. This factor appears to be distinct from other known inducers of vascular permeability. We are now in the process of purifying and characterizing this factor. Our long range goal is to better understand the regulation and mechanism of action of this factor in order to minimize the clinical complications.           </p>		



























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